Evidence Report:

Risk of Impaired Performance Due to Reduced Muscle Mass, Strength, and Endurance

Human Research Program
Human Health Countermeasures Element

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TABLE OF CONTENTS:

I. PRD Risk Title: Risk of Impaired Performance Due to Reduced Muscle Mass, Strength, and Endurance

Description: There is a growing research database that suggests that skeletal muscles, particularly postural muscles of the lower limb, undergo atrophy and structural and metabolic alterations during space flight. However, the relationships between in-flight exercise, muscle changes, and performance levels are not well understood. Efforts should be made to try to understand the current status of in-flight and post-flight exercise performance capability and what the goals/target areas for protection are with the current in-flight exercise program.

II. Executive Summary

This report reviews the scientific literature that documents the risk that exposure to the microgravity environment of spaceflight results in significant unloading of skeletal muscle, which in turn leads to loss of muscle mass (atrophy) and decrements in muscle strength and endurance. The chronological history of U.S. spaceflight is reviewed as a starting point to understand the current state of knowledge and the gaps in the knowledge base relevant to this risk. An overview of key scientific investigations that have been conducted before, during, and after human spaceflight, as well as human ground-based analog studies that contribute to the evidence base, is provided. Rodent and nonhuman primate experiments completed either during spaceflight or by means of ground-based flight simulations of skeletal muscle unloading are summarized. These animal studies have provided additional crucial information about this risk topic that can be extrapolated to human subjects to fill some of the knowledge gaps. Finally, the relationship of this risk to various spaceflight operational scenarios is examined and discussed.

III. Introduction

"Space flight investigations are essentially 'field studies,' fraught with many attendant difficulties, in which the investigator is even farther removed from experiment and subject than in field studies on Earth. . . . the circumstances fall short of the classical picture of the experimenting scientist in his exceptionally well equipped laboratory, constantly fine-tuning his equipment and personally conducting experimental trials and collecting precious data."

–Lawrence F. Dietlein, M.D., Ph.D., 1977

From the very beginning of the U.S. human space program, serious and reasonable concern has been expressed regarding exposure of humans to the microgravity of space due to the potential systemic effects on terrestrially evolved life forms that are so suitably adapted to Earth gravity. Humans in the microgravity environment of space, within our current space vehicles, are exposed to various mission-specific periods of skeletal muscle unloading (unweighting). Unloading of skeletal muscle, both on Earth and during spaceflight, results in remodeling of muscle (atrophic response) as an adaptation to the reduced loads placed upon it. As a result, decrements occur in skeletal muscle strength, fatigue resistance, motor performance, and connective tissue integrity. In addition, there are cardiopulmonary and vascular changes, including a significant decrease in red blood cell mass, that have an impact on skeletal muscle function. This normal adaptive response to the microgravity environment is, for the most part, of little consequence within the space vehicle *per se*, but may become a liability resulting in increased risk of an inability or decreased efficiency in crewmember performance of physically

demanding tasks during extravehicular activity (EVA) or abrupt transitions to environments of increased gravity (such as return to Earth or landing on the surface of another planetary body).

In the U.S. human space program, the only in-flight countermeasure to skeletal muscle functional deficits that has been utilized to date is physical exercise. In-flight exercise hardware and protocols have varied from mission to mission, somewhat dependent on mission duration and the volume of the spacecraft available for performing countermeasures. Collective knowledge gained from these missions has aided in the evolution of exercise hardware and protocols in attempts to refine the approach to prevention of spaceflight-induced muscle atrophy and the concomitant deficits in skeletal muscle function. Long-duration missions and exploration missions with several transitions between gravitational environments present the greatest challenges to risk mitigation and to development of countermeasures of proven efficacy. Russian scientists have utilized a variety of exercise hardware and in-flight exercise protocols during long-duration spaceflight (up to and beyond 1 year) aboard the Mir space station. On the International Space Station (ISS), a combination of resistive and aerobic exercise has been used. Outcomes have been acceptable according to current expectations for crewmember performance on return to Earth. However, for missions to the moon, establishment of a lunar base, and interplanetary travel to Mars, the functional requirements for human performance during each specific phase of these missions have not been sufficiently defined to determine whether currently developed countermeasures are adequate to meet physical performance requirements.

Access to human crewmembers during both short- and long-duration missions for the study of skeletal muscle adaptation to microgravity and the efficacy of countermeasures has been, and continues to be, limited. Consequently, a more complete understanding of physiological adaptations and protection against negative outcomes has required the use of ground-based models for the conduct of both fundamental and applied skeletal muscle research. Various models for which sufficient data have been collected have been concisely reviewed (Adams et al. 2003). Such models include horizontal or head-down bed rest, dry immersion bed rest, limb immobilization, and unilateral lower-limb suspension. While none of these ground-based analogs provides a perfect simulation of human microgravity exposure during spaceflight, each is useful for the study of particular aspects of muscle unloading as well as for investigation of sensorimotor alterations. Due to limitations in the number of spaceflights and crewmembers in which novel countermeasures can be tested, future development, evaluation, and validation of new countermeasures to the effects of skeletal muscle unloading will likely employ variations of these same basic ground-based models. Prospective countermeasures may include pharmacological and/or dietary interventions, innovative exercise hardware that provides improved loading modalities, locomotor training devices, passive exercise devices, and artificial gravity either as an integral component of the spacecraft or as a discrete device contained within it. With respect to the latter countermeasure, the hemodynamic and metabolic responses to increased loading provided by a human-powered centrifuge have been described recently (Caiozzo et al. 2004). Even more recently, an approach to provide both aerobic and resistive exercise by incorporating a cage-like platform into the design has been developed by the same investigator group.

Animal studies, conducted both during spaceflight and in ground-based simulations of the skeletal muscle unloading associated with spaceflight, have contributed to the scientific knowledge base in a manner not completely achievable by means of human spaceflight and ground-based analog studies alone. This is because many of the variables present with human subject investigations can be more tightly controlled in animal studies, and the much larger

number of animals typical of such experiments contributes to a greater statistical power to detect differences. A major advantage of the use of rodent models is that the adaptive changes to both spaceflight and hind-limb suspension occur in a much shorter time frame than they do in humans (hours to days versus days to weeks). This enables prediction of long-term changes in human skeletal muscle based on the shorter absolute time frame of the rodent investigations. Additionally, it is possible to perform a highly controlled, straightforward experiment in rodents without a requirement to provide some type of countermeasure intervention that introduces a confounding variable. In human studies, it is not possible on ethical grounds to withhold countermeasures known to have some degree of effectiveness to provide a population of true control subjects, in which only the effects of spaceflight are seen, for comparison with subjects utilizing countermeasure modalities. Animal studies do not suffer from such restrictions. Further work is needed to provide a better understanding of the problem, which will allow novel approaches to countering loss of skeletal muscle function associated with spaceflight in humans. Relevant animal spaceflight studies, as well as investigations using muscle unloading paradigms that contribute to our current knowledge base, are presented.

The purpose of this document is to provide a review of previous investigations and relevant data related to the risk of impaired performance due to reduced muscle mass, strength, and endurance associated with human spaceflight. To comprehensively assess this risk, it is important to have a thorough understanding of the evidence for functional deficits that occur in human crewmembers after spaceflight despite the performance of current countermeasures and, additionally, to fully define the mission-specific functional requirements. The inability of countermeasures to provide a level of skeletal muscle performance secondary to loss of muscle mass and strength, increased fatigability, and/or decrements in motor control needed to maintain crew health and safety and to meet both planned mission objectives and unforeseen contingencies defines the risk that must be mitigated. Evidence from over four decades of human spaceflight experience indicates that there are gaps in our knowledge and in our current approach to mitigate such risks. Although improvements have been made in the ability to maintain crewmember skeletal muscle performance, preservation of an appropriate level in every crewmember has not yet been achieved.

IV. Evidence

A. Human Spaceflight Evidence

1. Relevant Data from Mercury and Gemini Programs

Prior to the launch of the first American astronaut, suborbital flights of non-human primates (chimpanzees) demonstrated that launch and entry, as well as short-duration microgravity exposure, were all survivable events (Link 1965).

The initial biomedical problem faced by Project Mercury (which ran from 1959-1963) was establishment of selection criteria for the first group of astronauts. Medical requirements for the Mercury astronauts were formulated by the NASA Life Sciences Committee, an advisory group of distinguished physicians and life scientists. Final selection criteria included results of medical testing as well as the candidates' technical expertise and experience. Aeromedical personnel and facilities of the Department of Defense (DoD) were summoned to perform the stress and psychological testing of astronaut candidates. The screening and testing procedures defined for

the selection of Mercury astronauts served as the basis for subsequent selection of Gemini and Apollo astronauts when those programs were initiated.

While the Mercury flights were largely demonstration flights, the longest Mercury mission lasting only approximately 34 hours, Project Mercury clearly demonstrated that humans could tolerate the spaceflight environment without major acute physiological effects, and some useful biomedical information was obtained, which included the following (Johnston 1975):

- Pilot performance capability was unaltered by spaceflight.
- All measured physiological functions remained within acceptable normal limits.
- No signs of abnormal sensory or psychological responses were observed.
- The radiation dose received was considered insignificant from a medical perspective.
- Immediately after landing, an orthostatic rise in heart rate and drop in systemic blood pressure were noted, which persisted for 7 to 19 hours post-landing.

Because of the short mission durations of Project Mercury, there was little concern about loss of musculoskeletal function; thus, no exercise hardware or protocols were developed for use during flight. However, the selection criteria ensured that astronauts were in excellent physical condition before flight.

Biomedical information acquired during the Mercury flights provided a positive basis on which to proceed with the next step, the Gemini Program, which took place during the 20 months from March of 1965 to November of 1966. The major stated objective of the Gemini Program was to achieve a high level of operational confidence with human spaceflight. To prepare for a lunar landing mission, three major goals had to be realized, namely, [1] to accomplish rendezvous and docking of two space vehicles; [2] to perform extravehicular activities and to validate human life support systems and astronaut performance capabilities under such conditions; and [3] (germane to this report) to develop a better understanding of how humans tolerate extended periods of weightless flight exposure. Thus, Project Gemini provided a much better opportunity to study the effects of microgravity on humans. In the 14-day Gemini 7 flight, salient observations were undertaken to more carefully examine the physiological and psychological responses of astronauts as a result of exposure to spaceflight and the associated microgravity environment.

The Gemini Program resulted in approximately 2000 man-hours of weightless exposure of U.S. astronauts. Additional observations included the presence of post-flight orthostatic intolerance that was still present for up to 50 hours after landing in some crewmembers, a decrease in red cell mass of 5-20% from preflight levels, and radiographic indications of bone demineralization in the calcaneus. No significant decrements in performance of mission objectives were noted, and no specific measurements of muscle strength or endurance were obtained that compared pre-flight, in-flight, and post-flight levels.

2. Relevant Data from the Apollo Program

The major objective of the Apollo Program was the landing of astronauts on the lunar surface and their subsequent safe return to Earth. The Apollo (1968-1973) biomedical results were collected from 11 crewed missions that were completed within the five-year period of the Apollo Program, from pre-lunar flights (missions 7 through 10); the first lunar landing (mission 11), and five subsequent lunar exploratory flights (missions 12 through 17). Apollo 13 did not

complete its intended lunar landing mission because of a pressure vessel explosion in the Service Module. Instead, it returned safely to Earth after attaining a partial lunar orbit.

Essential to the successful completion of the Apollo Program was the requirement for some crewmembers to undertake long and strenuous periods of extravehicular activity (EVA) on the lunar surface. Naturally, there was concern about the capability of crewmembers to accomplish the lunar surface excursions planned for some of the Apollo missions. Although reduced lunar gravity was expected to make some tasks less strenuous, reduced suit mobility coupled with a complex and ambitious timeline led to the prediction that metabolic activity would exceed resting levels for extended periods. Because the nature and magnitude of physiological dysfunction resulting from microgravity exposure had not yet been established (and is still not concisely defined), suitable physiological testing was completed within the constraints of the Apollo Program to determine whether crewmember physiological responses to exercise were altered as a consequence of spaceflight.

Initial planning for the Apollo Program included provisions for in-flight measurements of salient parameters of concern, including physiological responses to exercise. However, the fire in the Apollo 204 spacecraft (also known as Apollo 1), fatal to astronauts Grissom, White, and Chaffee, resulted in the initiation of changes in the program by NASA management that eliminated such prospects. Thus, investigators were left with only the possibility to conduct pre-flight and post-flight exercise response studies and to assume that these findings reflected alterations of cardiopulmonary and skeletal muscle function secondary to microgravity exposure. It was realized early on that within the context and constraints imposed by the realities of the Apollo missions, the inability to control certain experimental variables would present challenges to many biomedical investigations. First, re-adaptation to Earth gravity begins immediately upon re-entry into the Earth's gravitational field, which likely changes key physiological responses from their measurements during spaceflight. Second, crew recovery procedures introduced additional challenges to a well-controlled experiment design, as Apollo crewmembers spent variable amounts of time in an uncomfortably warm spacecraft bobbing in the ocean, and additionally, orbital mechanics constraints on re-entry times imposed crew recovery times that prevented the possibility of conducting pre- and post-flight testing within a similar circadian schedule. The impact of these uncontrollable conditions and that of other physical and psychological stresses could not be separated from responses attributable to microgravity exposure alone. Thus, data related to the physiological responses to exercise stress in Apollo astronauts must be interpreted within this overall context.

No standardized in-flight exercise program was planned for any of the Apollo flights; however, an exercise device (Figure 1) was provided on some missions. Crewmembers, when situated in the Command Module (CM), typically used the exerciser several times per day for periods of 15-30 min.

The pre- and post-flight testing consisted of graded exercise stress tests conducted on a bicycle ergometer (Rummel and E.L. Michel 1975). Heart rate was used to determine stress levels (Maxfield and Brouha 1963), and the same heart rate levels were used for pre- and post-flight testing.

Figure 1. The exercise device used on some Apollo missions was based on the Exer-Genie developed by Exer-Genie, Inc., Fullerton, CA. Within the cylinder, the nylon cords rotate around a shaft, developing controlled resistance. The cords are attached to loop handles. When not in use, the flight device was stored in a cloth bag *(inset)*.

Although the exact duration of each stress level was adjusted slightly (1-2 minutes) for the later Apollo missions to obtain additional measurements, the graded stress protocol included exercise levels of 120, 140, and 160 beats per minute, corresponding to light, medium, and heavy work, respectively, for each individual. For the Apollo 9 and 10 missions, a stress level of 180 beats per minute was added. The entire test protocol was conducted 3 times within a 30-day period before lift-off. Post-flight tests were conducted on recovery (landing) day and once more at 24 to 36 hours after recovery.

During each test, workload, heart rate, blood pressure, and respiratory gas exchange (O_2 consumption, CO_2 production, and minute volume) measurements were made. For the Apollo 15 to 17 missions, cardiac output measurements were obtained by the single-breath technique (Buderer et al. 1973; Kim et al. 1966). Arteriovenous oxygen differences were calculated from the measured oxygen consumption and cardiac output data.

The data collected were voluminous and are summarized in tabular form by Rummel et al. (Rummel and E.L. Michel 1975). Dietlein has provided a concise synopsis of the findings (Dietlein 1975). In brief, reduced work capacity and oxygen consumption of significant degree was noted in 67% (18 of 27) of the Apollo crewmembers tested on recovery. This decrement was transient, and 85% of those tested (23 of 27) returned to preflight baseline levels within 24-36 hours. A significant decrement in cardiac stroke volume was associated with diminished exercise tolerance. It was not clear whether the exercise decrement had its onset during flight. If it did, the Apollo data did not reveal the precise in-flight time course because of lack of in-flight measurement capabilities. The astronauts' performance on the lunar surface provided no reason to believe that any serious exercise tolerance decrement occurred during flight, except that related to lack of regular exercise and muscle disuse atrophy (Dietlein 1975).

The studies completed during Apollo, although less than optimal, left no doubt that a decrement in exercise tolerance occurred in the period immediately after landing, although it is believed that such decrements were not present during surface EVA. It seems likely that multiple factors are responsible for the observed decrements. Lack of sufficient exercise and development of muscle disuse atrophy probably contributed. Catabolic tissue processes may have been accentuated by increased cortisol secretion as a consequence of mission stress and individual crewmember reaction to such stress. Additional factors associated with the return to Earth's gravity may also be implicated. Thus, the observed diminished stroke volume (cardiac output) is certainly contributory and, in turn, is a reflection of diminished venous return and contracted effective circulating blood volume induced by spaceflight factors (Dietlein 1975). Skeletal muscle atrophy is mentioned with respect to its possible contribution to exercise intolerance, and in some of the later Apollo flights, lower limb girth measurements were completed (data not published) that provided the first evidence for loss of muscle mass in the legs.

3. Relevant Data from the Skylab Program

The Skylab Program (May 1973-November 1974) was from the onset intended to provide a life sciences laboratory in space. A significant number of experiments were conducted to provide physiological data from humans exposed to long-duration stays in a microgravity environment.

A 56-day ground-based simulation of many of the Skylab experiments, conducted in an environmentally controlled, enclosed chamber, was termed the Skylab Medical Experiments Altitude Test (SMEAT) and represented the first mission. The three subsequent orbital missions were termed Skylab 2, 3, and 4. These three long-duration missions were 28, 56, and 84 days in duration, respectively. Collectively, the Skylab missions achieved a milestone in providing a vast array of human spaceflight biomedical information during missions of longer duration than any previous mission.

With respect to the current issue of loss of muscle mass and function, two key studies were performed during the course of the three Skylab orbital missions. First, leg and arm volumes were calculated by measuring the girth (circumference) of contiguous 3-centimeter arm and leg segments, with all the segments treated as a short tapered cylinder, and then summing the segment volumes to obtain the volume of each extremity.

The second study included the first muscle strength measurements by means of a dynamometer (Thornton 1977a; Thornton 1977b). In addition to measurements directly related to skeletal muscle strength and mass, indirect measurements were made that demonstrated that all Skylab crewmembers had a negative nitrogen balance (Whedon 1977) indicative of skeletal muscle attrition. This was also observed 10 years later in short-duration Space Shuttle crewmembers (Stein et al. 1996).

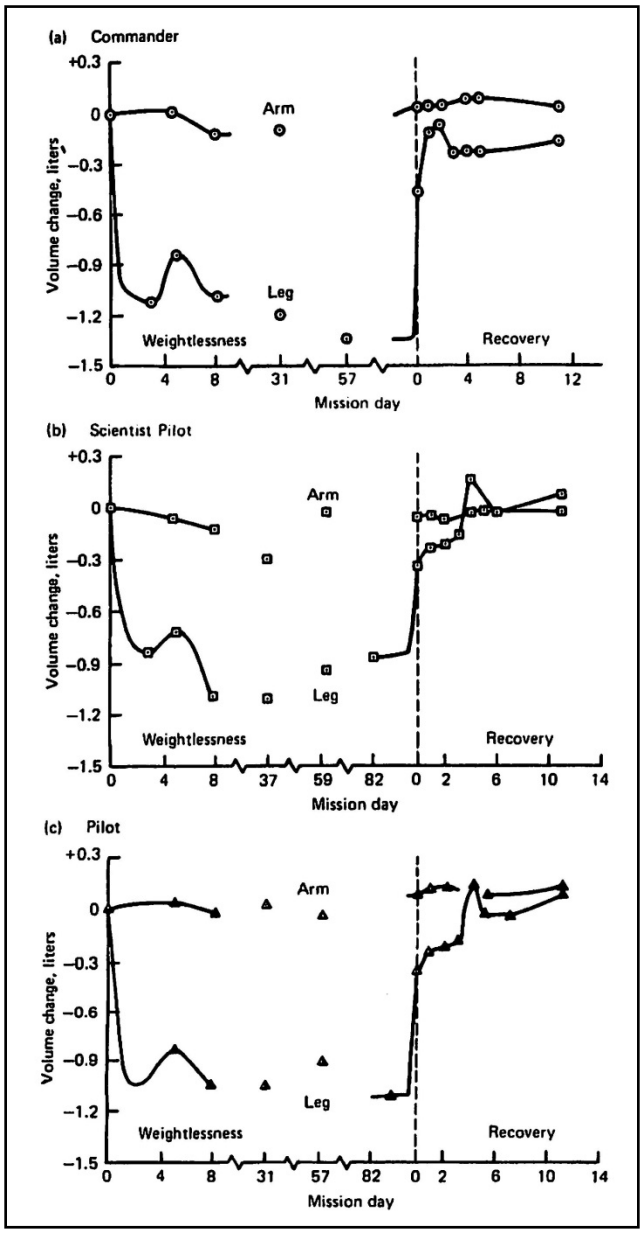

Figure 2. Changes in upper and lower limb volumes obtained by circumference measurements of 3-cm segments in the three crewmembers from Skylab 4. It should be noted that, because of a much higher exercise volume in the Skylab 4 crewmembers, their loss of muscle volume was much less than that observed in crewmembers from Skylab 2 and 3 (Thornton 1977a).

Upper and lower limb volumes of the three crewmembers of Skylab 4 are shown in Figure 2. Fluid shifts contributed the largest changes to lower limb volumes, but loss of leg tissue mass is clearly evident, particularly in the Commander. As shown in the graphs, significant loss of leg volume occurs within the first few days of microgravity exposure, while changes in the upper limbs are less remarkable. Upon return to Earth, much of the loss of leg volume is corrected and there is often a short overcorrection or overshoot. Once this fluid shift resolves, the true loss of

muscle mass remaining in the legs is revealed that more slowly returns to the baseline or preflight level (see Figure 2, leg during recovery on right side of graph for all three crewmembers).

In the Skylab 4 Commander, the loss in leg volume appears to be nearly 300 cc (Figure 2, topmost graph). Because the complement of exercise equipment for this mission was the largest (consisting of a cycle ergometer, passive treadmill, and the "Mini gym," modified commercial devices that provided the capability for low-load resistive exercises), losses in muscle mass and strength were less than those in the previous two missions of shorter duration.

During the Skylab Program, exercises and exercise devices were added incrementally and the testing expanded with each mission. This produced a different exercise environment for each flight so that in reality there were three separate but related orbital experiments, each with N = 3. The results from each mission had a significant impact on the next (Thornton 1977b).

Pre-flight and post-flight evaluations of muscle strength were performed on the right arm and leg of each crewmember for all three Skylab orbital missions by means of a Cybex isokinetic dynamometer (Thornton 1977b). The protocol completed on each crewmember included a thorough warm-up and 10 maximum-effort full flexions and extensions of the arm at the elbow and of the hip and knee at an angular rate of 45°/second. The isokinetic leg strength results from all three missions, as well as body weights and leg volumes, are presented in Figure 3.

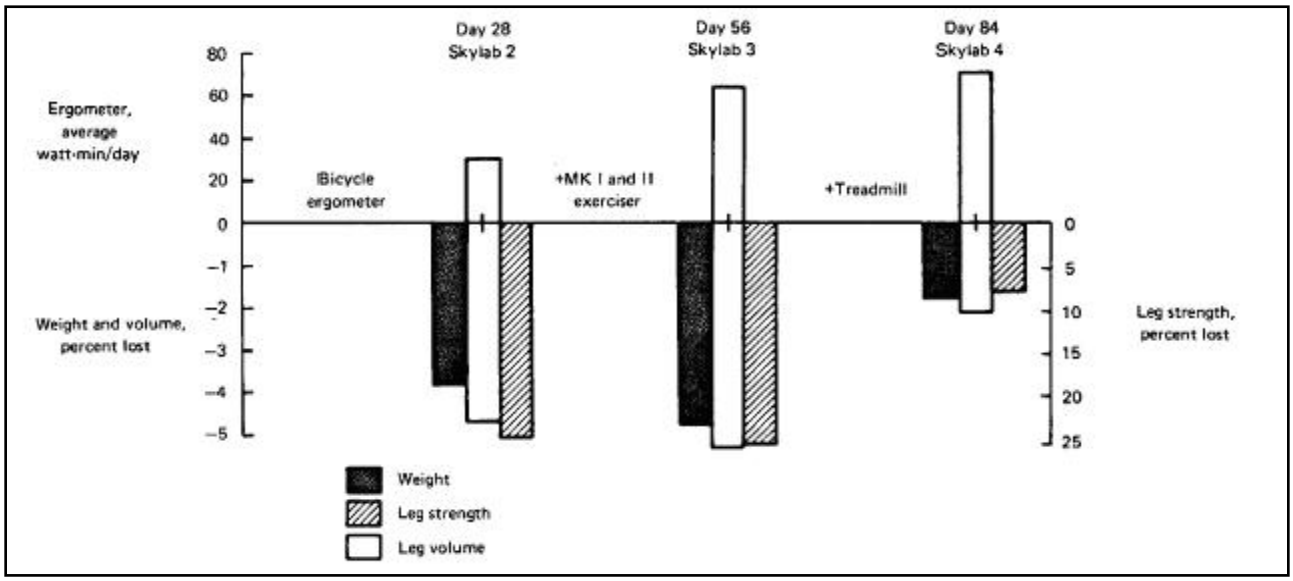

Figure 3. Average changes in body weight, isokinetic leg strength, and leg volume of crewmembers on the three Skylab missions. Only the bicycle ergometer was used on Skylab 2, the MK I and MK II "Mini Gym" exercisers were added for Skylab 3, and a passive "treadmill" was flown on Skylab 4. The average work load per day on the cycle ergometer is also provided by mission for comparison. From reference (Thornton 1977b).

On Skylab 2, only the bicycle ergometer was available for in-flight exercise, with testing performed 18 days before launch and 5 days after landing. While it was realized that these times were too temporally remote from the flight, this was the best that could be achieved due to schedule constraints. By the time day 5 of the muscle testing was completed, some recovery in function had likely occurred; however, a marked decrement still remained. The decrement in leg extensor strength was nearly 25%; the arms suffered less but also exhibited marked losses (data not shown). The Commander's arm extensors showed no loss, as he used these muscles in hand-

pedaling the bicycle, being the only Skylab crewmember to adopt this mode of arm exercise. This illustrates a fundamental point in muscle conditioning: to maintain the strength of a muscle, it must be stressed to or near the level at which it will have to function. Leg extensor muscles important in standing and providing propulsive forces during walking are capable of generating forces of hundreds of pounds, while the arm extensor forces are measured in tens of pounds. Forces developed while pedaling a bicycle ergometer are typically tens of pounds and are thus incapable of maintaining leg strength. The bicycle ergometer proved to be an excellent machine for aerobic exercise and cardiovascular conditioning, but it was not capable of developing either the type or level of forces needed to maintain strength for walking under 1 G (Thornton 1977b).

Immediately after Skylab 2, work was started on devices to provide adequate exercise to arms, trunk, and legs. A commercial device, termed "Mini Gym," was modified extensively and designated "MK-I." Only exercises that primarily benefited the arms and trunk were achievable with this device. While forces transmitted to the legs were greater than those from the cycle ergometer, they were still limited to an inadequate level, as this level could not exceed the maximum strength of the arms, which represents a fraction of leg strength (Thornton 1977b).

A second device, designated "MK-II," consisted of a pair of handles between which up to five extension springs could be attached, allowing development of maximum forces of 25 pounds per foot. These two devices were flown on Skylab 3, and in-flight nutrition support, exercise time, and food were increased. The crew performed many repetitions per day of their favorite maneuvers on the MK-I and, to a lesser extent, on the MK-II. Additionally, the average amount of work performed on the bicycle ergometer was more than doubled on Skylab 3, with all crewmembers participating actively.

It was perceived by Skylab life scientists that a device that allowed walking and running under forces equivalent to Earth gravity would provide more strenuous exercise (Thornton 1977b). Immediately after completion of Skylab 2, work was begun on a treadmill for Skylab 4. As mission preparation progressed, the launch weight of Skylab 4 escalated so much that the final design of the treadmill was constrained by weight limitations. The final weight for the device was a mere 3.5 pounds. This passive device (Figure 4) consisted of a Teflon-coated aluminum walking surface attached to the Skylab iso-grid floor. Four rubber bungee cords provided an equivalent weight of approximately 80 kilograms (175 lbs) and were attached to a shoulder and waist harness worn by crewmembers during use. By angling the bungee cords so that the user was pulled slightly forward, an equivalent to a slippery hill was created. High loads were placed on some leg muscles, especially in the calf, and fatigue was so rapid that the device could not be used for significant aerobic work because of the bungee/harness design. It was absolutely necessary to wear socks and no shoes to provide a low-friction interface with the Teflon surface.

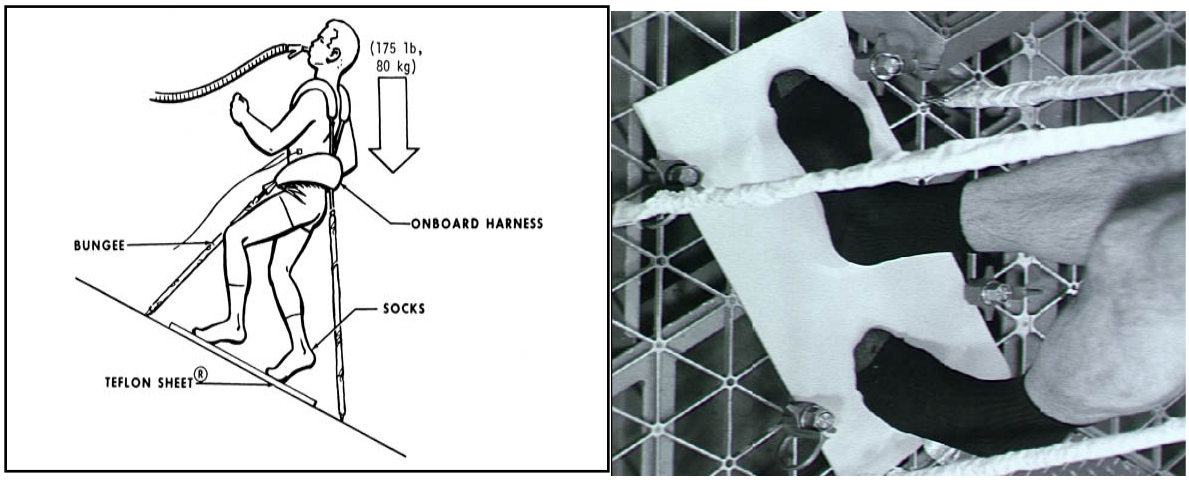

Figure 4. The first U.S. "treadmill" utilized during spaceflight was a passive device used only on the Skylab 4 mission with an 84-d duration. The high loading (175 lbs) via bungee cords provided more of a resistive rather than an aerobic modality. It consisted of a Teflon-coated aluminum plate attached to the Skylab iso-grid floor. The exercising crewmember wore a waist and shoulder harness that attached to the iso-grid floor surrounding the treadmill plate by means of 4 bungee cords. Socks had to be worn to provide a low-friction interface between the plantar surface of the feet and the Teflon-coated treadmill plate (Thornton 1977b).

On Skylab 4, the crew used the bicycle ergometer at essentially the same rate as that used on Skylab 3, as well as the MK-I and MK-II Mini Gym exercisers. In addition, they typically performed 10 minutes per day of walking, jumping, and jogging on the treadmill. Food intake had again been increased.

Upon their return to Earth and even before muscle testing, it was apparent that the Skylab 4 crewmembers were in very good physical condition. They were able to stand and walk for long periods without apparent difficulty on the day after landing (R+1), in contrast to the crewmembers from the earlier two missions. Results of strength testing confirmed a surprisingly small loss in leg strength even after nearly 3 months of microgravity exposure (Figure 3). In fact, knee extensor strength increased over the preflight level.

4. Relevant Data from the Space Shuttle Program

A variety of investigations related to skeletal muscle function have been completed during the course of the Space Shuttle Program. One of the most comprehensive of these was a suite of investigations accomplished during the Extended Duration Orbiter Medical Project (EDOMP), which was carried out during 1989-1995 with missions of up to 16 days. Studies most relevant to the risk on which this report focuses include the following: DSO 475 - Direct assessment of muscle atrophy and biochemistry before and after short spaceflight; DSO 477 - Evaluating concentric and eccentric skeletal muscle contractions after spaceflight; DSO 606 - Assessing muscle size and lipid content with magnetic resonance imaging after spaceflight; and DSO 617 - Evaluating functional muscle performance.

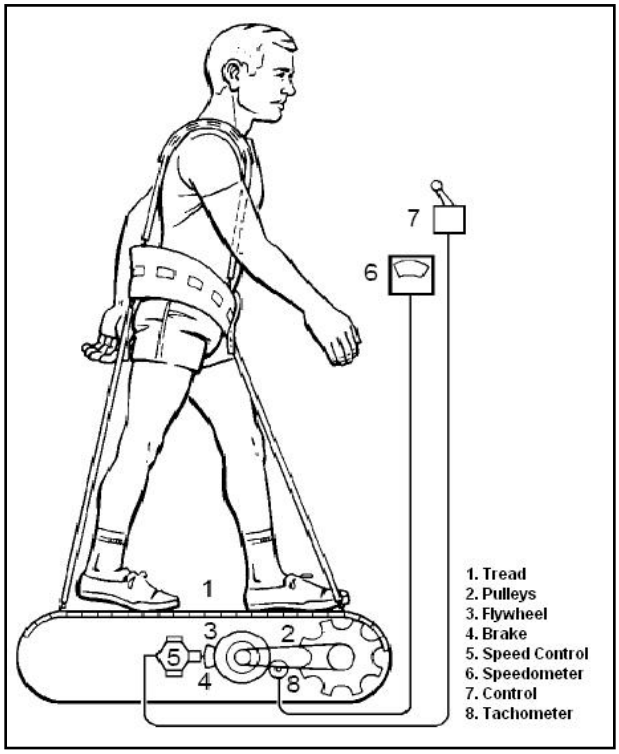

Figure 5. First-generation or original Space Shuttle passive treadmill (Greenisen 1999).

The collective specific aim of DSO 477 and DSO 617 was to evaluate functional changes in concentric and eccentric strength (peak torque) and endurance (fatigue index) of the trunk, arms, and legs of crewmembers before and after flight. LIDO® dynamometers located at the Johnson Space Center and at both the prime and contingency landing sites were used to evaluate concentric and eccentric contractions before and after flight.

Test subjects in this study exercised during flight for various durations, intensities, and numbers of days on the original Shuttle treadmill (Figure 5) (as opposed to the EDO treadmill, which flew on later Shuttle missions and was the basis for the ISS treadmill) as part of separate in-flight investigations. Exercise protocols included continuous and interval training, with prescriptions varying from 60% to 85% of preflight $VO_{2\text{-max}}$ as estimated from heart rate (HR). Some subjects had difficulty in achieving or maintaining their target HR during flight. The speed of this passive treadmill was controlled at seven braking levels by a rapid-onset centrifugal brake (see Figure 5). A harness and bungee/tether system was used to simulate body weight by providing forces equivalent to an approximate 1-G body mass. Subjects on this non-motorized treadmill were required to walk and run at a positive percentage grade to overcome mechanical friction. Study participants were familiarized with the LIDO® test protocol and procedures approximately 30 days before launch (L-30), after which time six test sessions were conducted. Three sessions were completed before launch (L-21, L-14, and L-8 days), and three were completed after landing (R+0, R+2, and R+7 to R+10 days).

The muscle groups tested are shown in Table 1. Torque and work data were extracted from force-position curves. Peak torque, total work, and fatigue index measured in the three pre-flight test sessions were compared; when no differences were found between sessions, values from the

three pre-flight sessions were averaged, and this average was used to compare pre-flight values with those on landing day and during the post-flight period.

Skeletal muscle strength was defined as the peak torque generated throughout a range of motion from 3 consecutive voluntary contractions for flexion and extension. Eccentric contractions are actions of the muscle whereby force is generated while the muscle is lengthening, as opposed to concentric actions characterized by muscle shortening (contracting) while generating force. Skeletal muscle endurance was defined as the total work generated during 25 repetitions of concentric knee exercise, as determined from the area under the torque curve for a complete exercise set. Work was also compared between the first 8 and last 8 repetitions. Endurance parameters were measured during concentric knee flexion and extension activity only. On R+0, significant decreases in concentric and eccentric strength were shown in the back and abdomen compared with the pre-flight means (Table 1).

Table 1. Mean percent change of skeletal muscle concentric and eccentric strength of various muscle groups on landing day compared with the pre-flight mean.

Muscle Group	*Test Mode*	
	Concentric	*Eccentric*
Back	–23 (± 4)*	–14 (± 4)*
Abdomen	–10 (± 2)*	–8 (± 2)*
Quadriceps	–12 (± 3)*	–7 (± 3)
Hamstrings	–6 (± 3)	–1 (± 0)
Tibialis Anterior	–8 (± 4)	–1 (± 2)
Gastroc/Soleus	1 (± 3)	2 (± 4)
Deltoids	1 (± 5)	–2 (± 2)
Pects/Lats	0 (± 5)	–6 (± 2)*
Biceps	6 (± 6)	1 (± 2)
Triceps	0 (± 2)	8 (± 6)

*Pre-flight > R+0 ($p < 0.05$); n=17.
Landing day (R+0) versus average of 3 pre-flight measures (Greenisen 1999).

Concentric back extension and eccentric dorsiflexion remained significantly less than pre-flight values on R+7. Recovery (an increase in peak torque from R+0 to R+7) was demonstrated for the eccentric abdomen and the concentric and eccentric back extensors.

However, the data depicted in Table 1 may be somewhat misleading because in some cases, there were significant differences in strength between crewmembers who exercised during flight versus those who did not. For example, some crewmembers who exercised during flight actually gained isokinetically measured strength in the ankle extensor/flexor muscles (anterior versus posterior calf muscles, i.e., *m. tibialis anterior* versus the gastrocnemius/soleus complex) compared with crewmembers who did not exercise and who actually showed a decrease in isokinetically measured strength in these muscles (Figure 6).

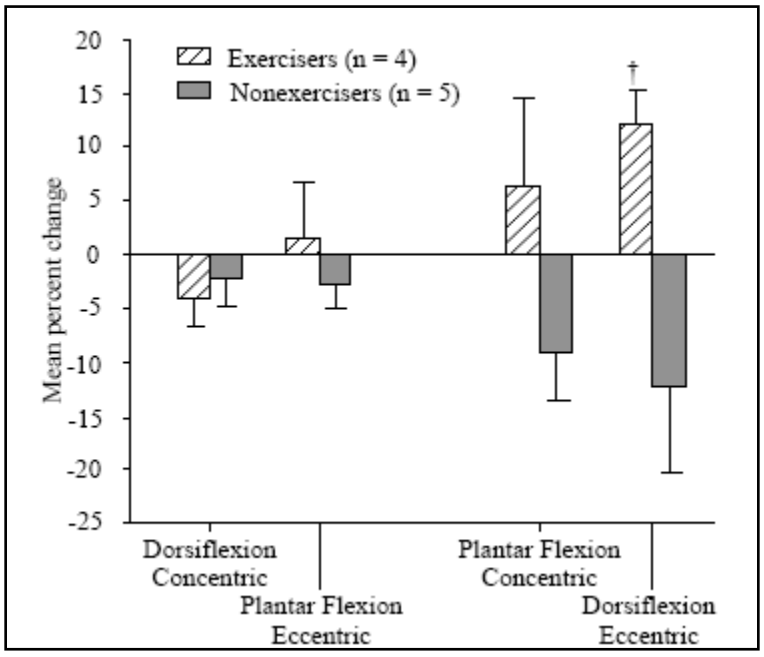

Figure 6. Percent change in isokinetic strength in ankle extensor and flexor muscles for crewmembers who exercised during flight versus those who did not. †Pre-flight < R+0 ($p < 0.05$) (Greenisen 1999).

With respect to endurance, the majority of the decrease in total quadriceps work occurred on R+0. This result likely reflects significant loss in the first third of the exercise bout (-11%). The declines in peak torque at the faster endurance test velocities are consistent with changes seen at the slower angular velocity used during the strength tests. Torque for the quadriceps at 75°/s was 15% less than preflight values but was 12% less than the pre-flight mean at 60°/s for the hamstrings. Endurance data showed little difference between pre-flight and R+7 test results, suggesting that crewmembers had returned to baseline by 1 week after landing.

Additionally, subjects who did exercise during flight compared with those who did not had significantly greater ($p < 0.05$) losses within 5 hours of landing in concentric strength of the back, concentric and eccentric strength of the quadriceps (30°/sec), and eccentric strength of the hamstrings relative to the respective preflight values (Greenisen 1999) (data not shown here). According to Greenisen et al., non-exercisers also had significantly less concentric strength of the quadriceps at 75°/s and lower total work extension, work first-third flexion, and work last-third extension immediately after landing than before flight. The conclusions reached by the investigators were that the data indicate that muscles are less able to maintain endurance and resist fatigue after spaceflight, as well as that exercise may prevent decrements in these aspects of endurance (Greenisen 1999).

Conversely, crewmembers who exercised during flight had greater losses in trunk muscle strength measured at landing compared with the non-exercising group (Figure 7). However, pre-flight strength in trunk flexion and extension was substantially greater in the exercising group than in the non-exercising group. Apparently, treadmill exercise did not prevent decrements in trunk strength after 9-11 days of spaceflight, and the investigators offered the explanation that preservation of muscle function may be limited only to those muscles that are effectively used as part of the exercise regimen.

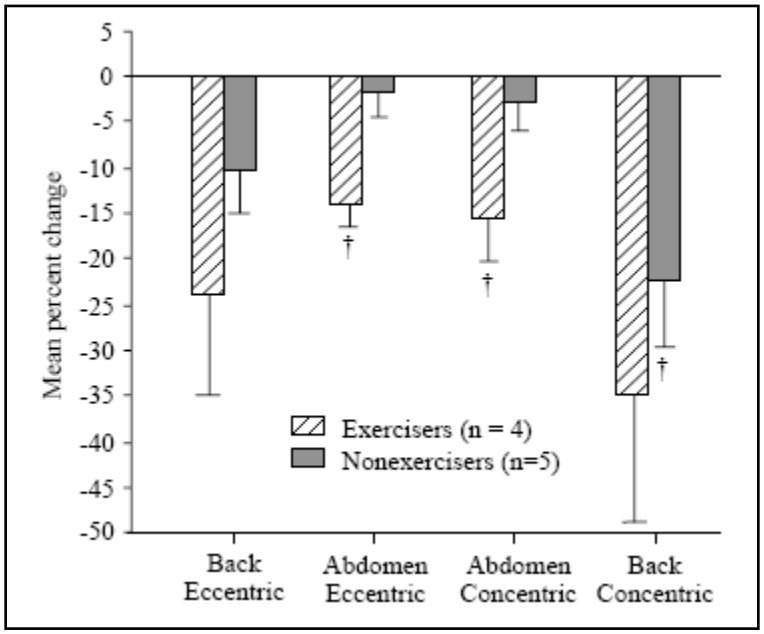

Figure 7. Percent change in isokinetic strength in trunk muscles in crewmembers who exercised during flight versus those who did not. †Pre > R+0 ($p < 0.05$) (Greenisen 1999).

The specific aim of DSO 475, "Direct Assessment of Muscle Atrophy Before and After Short Spaceflight," was to define the morphological and biochemical effects of spaceflight on skeletal muscle fibers (Greenisen 1999). To obtain myofiber biochemical and morphological data from Space Shuttle crewmembers, biopsies were conducted once before flight (L->21 days) and again on landing day (R+0). The subjects were eight crewmembers, three from a 5-day mission and five from an 11-day mission. Biopsies of the mid-portion of the *m. vastus lateralis* were obtained by means of a 6-mm biopsy needle with suction assistance. Muscle fiber cross-sectional area (CSA), fiber distribution, and number of capillaries were determined for all crewmembers before and after flight.

The CSAs of slow-twitch (Type I) fibers in post-flight biopsies were 17% and 11% lower than those in pre-flight biopsies for 11- and 5-day flyers, respectively (Edgerton et al. 1995). Similarly, the CSAs of fast-twitch (Type II) fibers were 21% and 24% lower in post-flight compared with pre-flight biopsies for 11- and 5-day flyers. Due to the extremely small sample sizes, these numbers do not reflect significant differences but nevertheless provide evidence that space flight-induced muscle atrophy occurs at the cellular level. Interestingly, when samples were further analyzed for changes in Type II sub-types, significant CSA reductions were detected in Type IIA (-23%) and Type IIB (-36%) fibers from crewmembers involved in the 11-day mission. The relative proportions of the Type I and Type II fibers were different before and after the 11-day mission; the fiber distribution followed the same trend after the 5-day mission (increased Type II and decreased Type I fibers compared with pre-flight), but the sample size was too small to reach statistical significance. This shift is consistent with the observed reduction in the number of individual muscle fibers that expressed the Type I myosin heavy chain protein (Zhou et al. 1995).

While no specific enzymatic activities involved in energy metabolism were found to be significantly different in muscle biopsy samples from returning crewmembers, the glycolytic/oxidative enzyme ratio of α-glycerophosphate dehydrogenase/succinate dehydrogenase activity was found to be increased (Edgerton et al. 1995), suggesting a shift resulting in decreased oxidative and increased glycolytic capacity in muscle fibers. The implication of such a shift is the potential of reduced fatigue resistance of the muscle during work. The number of capillaries per fiber was significantly reduced after 11 days of spaceflight. However, because the mean fiber size was also reduced, the number of capillaries per unit of CSA of skeletal muscle tissue remained the same (Edgerton et al. 1995). Atrophy of both major myofiber types, with atrophy of Type II > Type I, is somewhat different from the more selective Type I myofiber atrophy observed in unloaded Sprague-Dawley and Wistar rat muscle (Itai et al. 2004; Jaspers and Tischler 1984; Steffen et al. 1990), representing an uncommon case in which differences exist between responses of human and murine skeletal muscle.

The purpose of DSO 606, "Quantifying Skeletal Muscle Size by Magnetic Resonance Imaging (MRI)," was to non-invasively quantify changes in size, water, and lipid composition in antigravity (leg) muscles after spaceflight. This experiment was the first attempt to measure limb volumes before and after flight since the less sophisticated methods of measuring limb girths during the Apollo and Skylab programs were used. The subjects included four Space Shuttle crewmembers from an 8-day mission. All subjects completed three pre-flight tests and two post-flight tests at R+1 and R+15/16. Testing involved obtaining a 1.5-Tesla MRI scan of the lower body. Multi-slice axial images of the leg were obtained to identify and locate various muscle groups. Muscle volumes for the calf, thigh, and lumbar regions were measured to assess the degree of skeletal muscle atrophy. Significant reductions were observed in the anterior calf muscles (-3.9%), the gastrocnemius/soleus muscles (-6.3%), hamstrings (-8.0%), and intrinsic back muscles (-10.3%). After two weeks of recovery, some residual atrophy still persisted. These whole muscle measures along with the cellular measurements clearly established that muscle atrophy begins rapidly in the unloaded environment of space and accounts, at least in part, for the observed losses in muscle strength.

The EDOMP provided significant knowledge on the effects of spaceflight on human physiology and, specifically, on alterations in skeletal muscle mass, strength, and function. Once again, losses of skeletal muscle mass, strength, and endurance were documented, despite the use of exercise countermeasures in some cases. However, some findings were encouraging,

particularly indications that in-flight exercise does have a positive effect in countering losses in muscle strength at least in the legs (see Table 1 and Figure 6), as predicted from the results of the 84-day Skylab 4 mission when multiple modes of exercise were used, including a unique "treadmill" device (see Figure 4). This unusual treadmill provided loads of sufficient magnitude to the legs in a manner approaching resistance exercise. However, the data provided by MRI volume studies indicate that not all crewmembers, despite utilization of various exercise countermeasures, escape the loss in muscle mass that has been documented during most of the history of U.S. human spaceflight since Project Mercury.

In addition to the EDOMP, the Life and Microgravity Spacelab (LMS) experiments represent another hallmark Space Shuttle Program initiative to better understand the physiological adaptations to spaceflight. LMS was conducted aboard STS-78 and involved four crewmember subjects who participated in each of the following muscle physiology studies during their 17-day mission.

Studies of muscle function and physiology. Muscle atrophy was assessed during LMS by MRI using procedures similar to those used for STS-47 (LeBlanc et al. 1995). Post-flight muscle volumes were significantly reduced (7-12%) in back muscles, quadriceps, gastrocnemius, soleus, and gluteal muscles on landing day (LeBlanc et al. 2000; Tesch et al. 2005). By R+10, all changes in muscle volume had reverted to pre-flight levels. The observed reductions in gastrocnemius, soleus, and quadriceps muscles following the 17-day LMS mission were on average larger than those reported for the 8-day STS-47. The MRI results not only directly confirm that muscle atrophy is an early consequence of space flight, but they also suggest that muscle atrophy continues during longer exposures to microgravity.

Whole muscle strength was measured in knee extensors and plantar flexors during LMS. The production of force by knee extensors was determined under isoinertial and isometric conditions (Tesch et al. 2005). Pre-flight and post-flight measurements were obtained with an instrumented leg press device that uses inertial flywheels as the resistance mode. The device could also be locked in place at a 90-degree knee angle for the measurement of maximal isometric force. Consistent with the reported reduction in quadriceps CSA, knee extensor (leg press) strength was reduced post-flight (R+1). Maximal isometric force was reduced by 10.2%, whereas concentric and eccentric strength were reduced by 8.7% and 11.5%, respectively.

In separate experiments involving the same astronaut subjects, calf muscle performance was assessed before, during, and after STS-78 with a torque-velocity dynamometer (TVD) (Trappe et al. 2001). The TVD was a mission-specific piece of hardware that measured ankle plantar flexion and dorsiflexion strength under isometric or isokinetic (fixed angular velocity) conditions. Angle-specific tests for isometric strength (80, 90, 100 degrees), isokinetic strength at speeds from 30-360 degrees/seconds, and isokinetic endurance were performed before, during and post-flight. In-flight tests were conducted on flight day (FD)2/3, FD8/9, and FD12/13. Post-flight tests were performed on R+2 and R+8. Muscle strength values were reported to be ~50% lower at the first two in-flight time points, but the charges were attributed to issues with the system that secured the TVD in place. The TVD was reported to be "lifting and floating" during testing. The issue was resolved prior to FD12/13 testing, at which time no differences in torque generation compared with pre-flight values were observed. Likewise, post-flight values were not

significantly different than pre-flight values. The authors of the investigation have suggested that the lack of change during 17 days of space flight may have been due to the nature in which the testing was conducted; that is, the in-flight testing may have served as an unexpected, yet effective, exercise countermeasure to protect the calf muscle from strength loss. The three in-flight calf muscle test sessions during STS-78 involved making ~525 calf muscle contractions on the TVD (Trappe et al. 2001), half of which were made at 80% to 100% of each individual's maximal values (Trappe 2002; Trappe et al. 2001). In contrast, the same LMS crew displayed significant deficits in both size and strength of the quadriceps (Tesch et al. 2005), a muscle group that was not tested during flight. This result suggests that high-intensity muscle contractions, which are performed less than daily, may protect muscle strength during missions of up to 17 days.

Loss of skeletal muscle strength is a consequence not only of reduced muscle size, but also of decreased neural drive and myocellular damage. Studies were performed on the calf muscles (contralateral leg to that used in studies described above) before flight, during flight (four time points), and after flight to separate the causal effects of muscle atrophy from reduced neuromuscular recruitment (Narici et al. 2003) to address this question. Surface electrodes were placed over the subjects' gastrocnemius and soleus, and a percutaneous electrical muscle stimulator (PEMS) unit was used to directly cause forced whole-muscle contractions independent of any voluntary input provided by the crew member. No measureable losses in electrically evoked calf muscle performance were observed. However, post-flight (R+8) reductions in force production were observed. Given the lack of change during late in-flight testing (FD16), it was suggested that alterations are likely due to muscle damage due to gravitational reloading of the muscles during normal ambulation. This notion was supported by MRI analyses. MRI transverse relaxation time (T2) of skeletal muscle is an indicator of increased tissue fluid volume and can be a marker of myocellular damage (inflammation/edema). In these crewmembers, T2 values were elevated at R+2 and stayed elevated at R+10.

Studies of muscle morphology and cellular function. Muscle biopsy samples were obtained from the 4 LMS crew members who participated in the whole-muscle size and function testing (Riley et al. 2000; Riley et al. 2002; Trappe et al. 2001; Widrick et al. 1999; Widrick et al. 2001). Biopsies were obtained from the gastrocnemius and soleus muscles before flight and again within three hours of landing. Functional analyses of single muscle fibers provide the most direct evidence of space flight-induced changes in the function of the muscle mechanics without the influence of factors such as changes in neuromuscular recruitment patterns or differences in volitional effort. Using calcium-activated individual muscles, any observed alterations in mechanics can be attributed to alterations in the myofiber itself. Individual muscle fibers from the LMS crew were isolated and mounted between a force transducer and a servomotor for analyses. Space flight produced a small decrease (-6%) in type I single-fiber peak calcium-activated force production (P_o) in samples from the gastrocnemius (Widrick et al. 2001). However, no difference was observed when these measurements were corrected for muscle fiber CSAs. No mean differences were found in P_o or fiber CSA for fibers that either expressed type IIa myosin heavy chain (MHC) or co-expressed both type IIa and IIx MHC. While mean differences in fiber mechanics were not observed in subjects as a group, significant changes occurred within individual subjects when subject-by flight analyses were conducted (each

subject had a cohort of fibers that were analyzed). In one subject, P_o and CSA in Type IIa fibers were reduced by 19% and 12%, respectively. In another subject, P_o was reduced by 23% in Type I fibers and 15% in Type IIa fibers, with reductions in fiber CSA of 7% for type I and 12% for type IIa. The investigators point out that the variability in space flight response seems to result, at least in part, from initial fiber size. Fibers with the greatest reduction in size and P_o tended to come from the crew members who had larger pre-flight fibers.

In the soleus muscle, a calf muscle adjacent to the gastrocnemius but one that is more slow and oxidative in nature, 91% of muscle fibers expressed only type I MHC before flight (Widrick et al. 1999). After space flight, the number of Type I fibers decreased to 79%. Space flight also resulted in a 21% decrease in mean P_o. This decline in Ca^2-activated peak force was paralleled by a 15% decrease in fiber CSA, which indicates that muscle atrophy accounted for most of the loss of function, although a 4% residual loss of P_o remained when P_o was normalized by individual fiber CSA.

Skeletal muscle power is generally viewed as a functional measure of muscle performance because, like most physical tasks that require high levels of exertion, peak values actually occur at submaximal loads. The power of single fibers was measured in a manner similar to the P_o measurements; however, instead of the measures being isometric, they were obtained with isotonic load clamps. No significant main effect of space flight was found on muscle power for single fibers from either the gastrocnemius (Widrick et al. 2001) or the soleus (Widrick et al. 1999) muscles. Despite some variability among crew members in the effect of space flight on P_o in various muscle fiber types, the overall trend showed that increases in maximal shortening velocity (V_o), which are attributed to decreased thin filament density based on observations from electron microscopy (Riley et al. 2000; Riley et al. 2002), compensate for the loss of P_o to maintain muscle power at the cellular level.

Skeletal muscle is a highly metabolic tissue. As is true for muscle size, the intensity and volume of physical activity are also major determinants of the readily adaptable bioenergetic capacity and composition of the muscle. Portions of the biopsy specimens from the gastrocnemius and soleus were used to perform biochemical analyses of oxidative and glycolytic enzymes. Despite some evidence of a metabolic shift toward glycolysis-derived energy sources in biopsy samples after the 11-day STS-32 mission (Edgerton et al. 1995), no differences were detected in citrate synthase, phosporylase, or β-hydroxyacyl-CoA dehydrogenase in samples after the 17-day LMS mission (Trappe et al. 2001). Accordingly, no post-flight changes were observed in muscle glycogen content. Therefore, while space-flight appears to promote a slow-to-fast shift in MHC, there does not appear to be a similar systemic metabolic shift.

5. Relevant Data from the Shuttle-Mir and NASA-Mir Programs

During the seven NASA-Mir flights, seven U.S. astronauts trained and flew jointly with 12 Russian cosmonauts over a total period of 977 days (the average stay was 140 days) of spaceflight, which occurred during the period from March 1995 to June 1998. The major contribution of the joint U.S./Russian effort on the Mir space station relevant to the current risk topic was the first use of MRI to investigate volume changes in the skeletal muscles of astronauts

and cosmonauts exposed to long-duration spaceflight. This began with the first joint mission, Mir-18, and continued until the final Mir-25 mission. The data indicated that loss of muscle volume, particularly in the legs and back, was greater than that in short-duration spaceflight but not as great as the data from short-duration flight may have predicted (LeBlanc et al. 2000). A comparison between volume losses in the selected muscle groups in short-duration spaceflight on the Space Shuttle, long-duration (119 d) bed rest, and a (115 d) Shuttle-Mir mission demonstrates the relative time course of the losses (Figure 8).

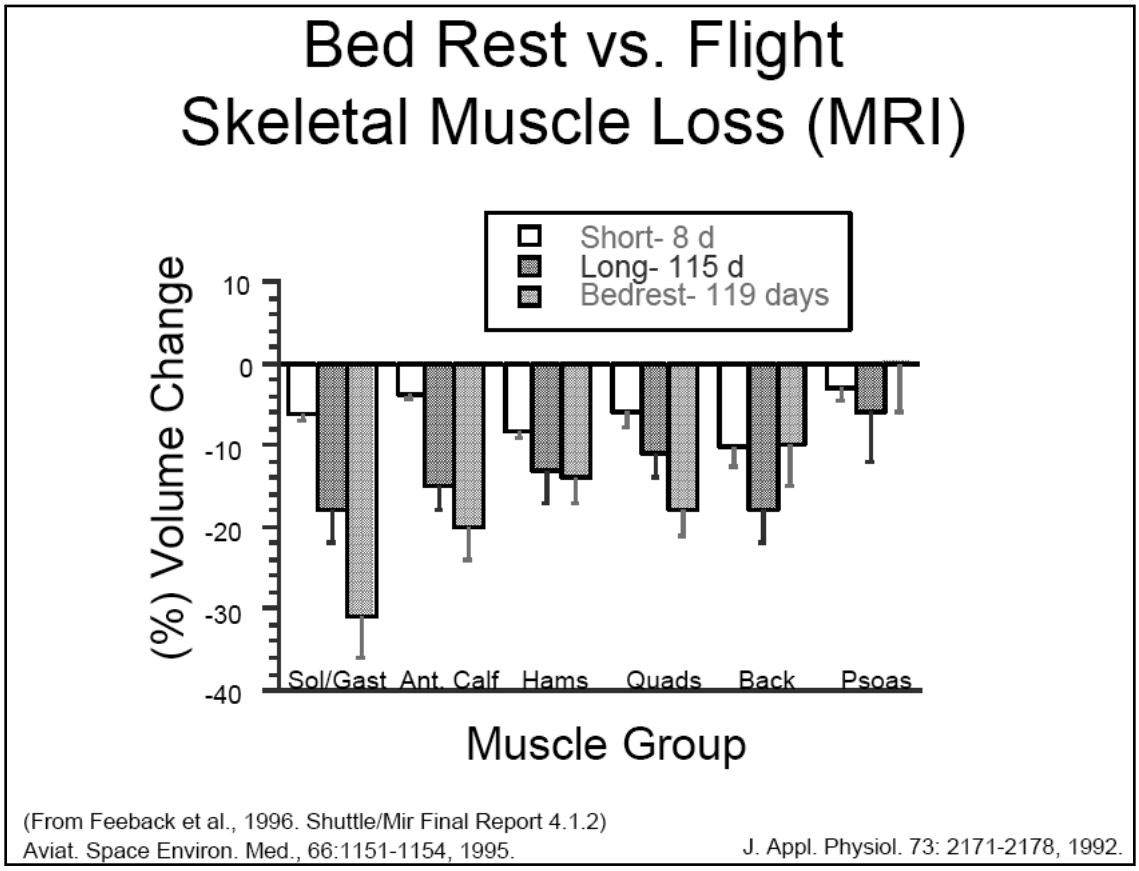

Figure 8. Percent change in selected muscle groups during short-duration (8 d; n = 8) and long-duration (115 d; n = 3) spaceflight (Mir 18) compared with long-duration bed rest (119 d). Data are from (LeBlanc et al. 1995; LeBlanc et al. 1992) and the Shuttle/Mir Final Report.

There is a good correlation between long-duration bed rest and spaceflight of similar duration except that losses in the back muscles are much lower with bed rest. This result likely reflects the use of these muscles during bed rest to adjust body position and to reduce the potential for vascular compression and tissue injury. During spaceflight, the back muscles are apparently less used because they do not have to support the upright body against Earth's gravity and are not used with great force to make positional adjustments of the body as they are during the recumbency of bed rest.

6. Relevant Data from the International Space Station (ISS) Program

The first ISS crew (Expedition 1) arrived in October 2000; since then, there have been 40 Increments. Two major research study complements addressing the *Risk of Impaired Performance Due to Reduced Muscle Mass, Strength, and Endurance* were conducted during the early phase of ISS exercise countermeasures evaluation. During these complements, subjects had access to the CEVIS cycle ergometer, the TVIS treadmill, and, importantly, the interim Resistive Exercise Device (iRED). iRED was an elastomer-based piece of resistance exercise hardware. This device was limited to a 300-pound maximum load. By comparison, the currently available ARED has a 600 pound load capacity. One investigation during the "iRED era" involved four ISS astronauts with mission durations of 161-194 days (Gopalakrishnan et al. 2010), and the other studied 10 astronauts and cosmonauts whose mission durations spanned a very similar 161-192 days in space (Fitts et al. 2013; Fitts et al. 2010a; Trappe et al. 2009). Each of these studies investigated changes in muscle size and strength, with one focusing on a larger array of muscle groups and the other performing a diverse set of whole muscle, cellular, and biochemical measures on the postural muscles of the calf.

Initial post-landing MRI data for both studies were obtained on a relatively similar timeline (5 ± 1 and 4 ± 1 days). Calf muscles were found to undergo greater decrements than thigh muscles (10-18% and 4-7% loss, respectively) (Gopalakrishnan et al. 2010). Both studies reported the greatest loss in the soleus muscle (%) with less, but substantial, decrements in the gastrocnemius (Gopalakrishnan et al. 2010; Trappe et al. 2009). Approximately half of the loss of muscle mass still existed up to two weeks following return to Earth (Trappe et al. 2009). Although these MRI results highlighted a clear need for improved countermeasures hardware and/or strategies, they also demonstrate an incremental improvement in the countermeasures targeted to mitigating muscle loss compared with the more dramatic reductions observed during Shuttle-Mir missions (LeBlanc et al. 2000). Muscle strength measurements in ISS crew members were not measured until approximately one week following landing. Nonetheless, strength losses accompanied muscle atrophy in both upper (Gopalakrishnan et al. 2010) and lower (Gopalakrishnan et al. 2010; Trappe et al. 2009) leg muscles. Isokinetic strength measures in thigh knee extensor muscles revealed a 10% loss (Gopalakrishnan et al. 2010), whereas calf muscle strength was reduced by 24% (Gopalakrishnan et al. 2010; Trappe et al. 2009), again demonstrating that the calf muscles are most susceptible to space-flight-induced decrements. The drop in torque production of the calf muscles was observable across the entire range of speeds used from 0-300 degrees/second (Trappe et al. 2009). This reduction in calf muscle performance, initially measured one week post-landing, persisted until at least two weeks after return despite a partial restoration in muscle volume (Trappe et al. 2009). Taken together, the results suggest that impairments in muscle strength are likely perturbed by muscle damage and/or soreness derived from gravitational reloading of the muscles.

Various structural and functional analyses were performed on muscle biopsy samples from the gastrocnemius and soleus muscles from nine ISS crewmembers (Fitts et al. 2010a; Trappe et al. 2009). Mirroring what was observed at the whole-muscle level, individual muscle fiber analyses also revealed muscle atrophy at the cellular level (Fitts et al. 2010a). Cross-sectional areas were determined in individual muscle fibers that were set at a standardized sarcomere length. The number of slow type I muscle fibers was reduced by 24% and 33% in the gastrocnemius and soleus muscles, respectively. The number of fast type II fibers (of all sub-types, excluding hybrids) was also reduced in the soleus muscle (29%) but was unchanged in the

gastrocnemius. Measures of muscle fiber mechanics clearly demonstrated decrements of function at the cellular level (Fitts et al. 2010a). Peak calcium activated force, maximal shortening velocity, and peak power were all markedly reduced in post-flight samples taken from gastrocnemius and soleus muscles, with the most dramatic change being a 45% loss of power production in type I soleus muscles. This is in stark contrast to responses to short-term Space Shuttle flights, where increases in maximal shortening velocity were able to compensate for reduced force production to maintain peak power levels. Power was also reduced in type II fibers, with reductions to maximal shortening velocity and peak force being contributing factors for fibers from gastrocnemius and soleus muscles, respectively.

In both gastrocnemius and soleus muscles, a clear shift in the contractile machinery was observed with a slower-to-faster phenotype reported (Trappe et al. 2009). This can be observed from MHC protein expression in the individual fibers that were analyzed for contractile properties. Both gastrocnemius and soleus muscles exhibited reductions in the amount of fibers expressing type I MHC. This corresponded to increases in the percentages of type IIa fibers and type I/IIa hybrid fibers from gastrocnemius muscle. A similar pattern occurred in the soleus muscle, although increases were primarily observed in the various hybrid fibers distributed in a manner such that significant changes were only detected in hybrid fibers grouped together.

Although limitations in the availability and accuracy of iRED loading data prevented investigators from making meaningful analyses of the relationships between resistance training loads and muscle adaptions during these ISS missions, a number of observations were made regarding treadmill running and alterations in the calf muscles (Trappe et al. 2009). Treadmill use ranged from less than 50 minutes a week to greater than 300 minutes per week. Subjects who ran on the treadmill the most preserved muscle better than those who ran less. When total aerobic exercise (TVIS treadmill + CEVIS bicycle ergometer) was compared with changes in muscle volume, this correlation was lost. Data demonstrating that foot forces are much higher during treadmill running versus cycling aboard ISS (Genc et al. 2010) support the argument that higher forces are vital to protecting against muscle atrophy during spaceflight. Results for treadmill use were not restricted to in vivo whole-muscle observations. Subjects who used the TVIS treadmill more than 200 minutes per week generally fared better than those who ran less than 100 minutes per week in terms of single fiber CSA, peak force, and power (Fitts et al. 2010a).

In addition to muscle mass and the function of the cellular contractile proteins, changes to the molecular mechanisms that control energy metabolism also have the potential to negatively affect human performance following exposure to long-duration space flight. Activities of a battery of oxidative and glycolytic enzymes were therefore measured in crewmembers before and after ISS missions (Fitts et al. 2013). Overall, the observed spaceflight effects on metabolic enzymes in skeletal muscle were minimal. No changes in activities of citrate synthase, β-hydroxyacyl-CoA, lactate dehydrogenase, or phosphofructokinase were observed in calf muscles following 6 months aboard ISS. Rather, spaceflight and exercise countermeasures play a more limited role in select adaptions to metabolic enzymes in calf skeletal muscles. For example, the mitochondrial enzyme cytochrome oxidase was reduced in spaceflight by 35% in type I soleus muscle in all crewmembers studied. However, this result was entirely accounted for by the crewmembers in the low treadmill use group (less than 100 minutes/week), in which a 59% reduction occurred. Activity levels in the high treadmill use group were unchanged. In short, metabolic adaptations in skeletal muscle appear to be less sensitive to unloading compared with structural and functional changes related to morphology and contractility. Furthermore, countermeasure strategies that are insufficient to fully protect muscle from unloading-induced

atrophy appear to be more effective in protecting against changes to the metabolic phenotype of the muscle.

These two major studies point to the need for high load intensity if prevention of muscle mass and strength is to be accomplished. In these early years, both hardware capabilities and reliability certainly contributed to this condition not being met. The iRED science requirement was to provide a load of up to an equivalent of 600 lb (273 kg); however, as mentioned above, the delivered hardware product provided only approximately half of that amount. Ground-based studies have shown that it does produce a positive training effect similar to that of equivalent free weights when used in a high-intensity program (Schneider et al. 2003), but it will likely not provide sufficient loading in a zero-gravity environment to prevent loss of muscle and bone tissue, as determined from parabolic flight studies (Lee et al. 2004). For whole-body resistance exercises, such as squats, one's own body weight contributes a significant amount of load in a 1-G environment. In the weightlessness of space, this contribution is lost. For this reason, load capacities for resistance exercise devices for use in space must be able to replace the body loads that are lost in the microgravity environment on top of the normal loads that one would use on the ground. Other problems in meeting load requirements were related to failures of the onboard exercise hardware with reduced utilization at other times, as well as use restrictions imposed due to transmission of forces into the structure of the space station itself. In fact, during the first eleven ISS Expeditions, there were only two short periods during Expeditions 3 and 4 when all three U.S. onboard exercise devices (CEVIS, TVIS, and iRED) were capable of being used under nominal conditions (Figure 9). The almost continuously suboptimal availability of exercise equipment likely has had a negative impact on maintenance of crew physical fitness during this time.

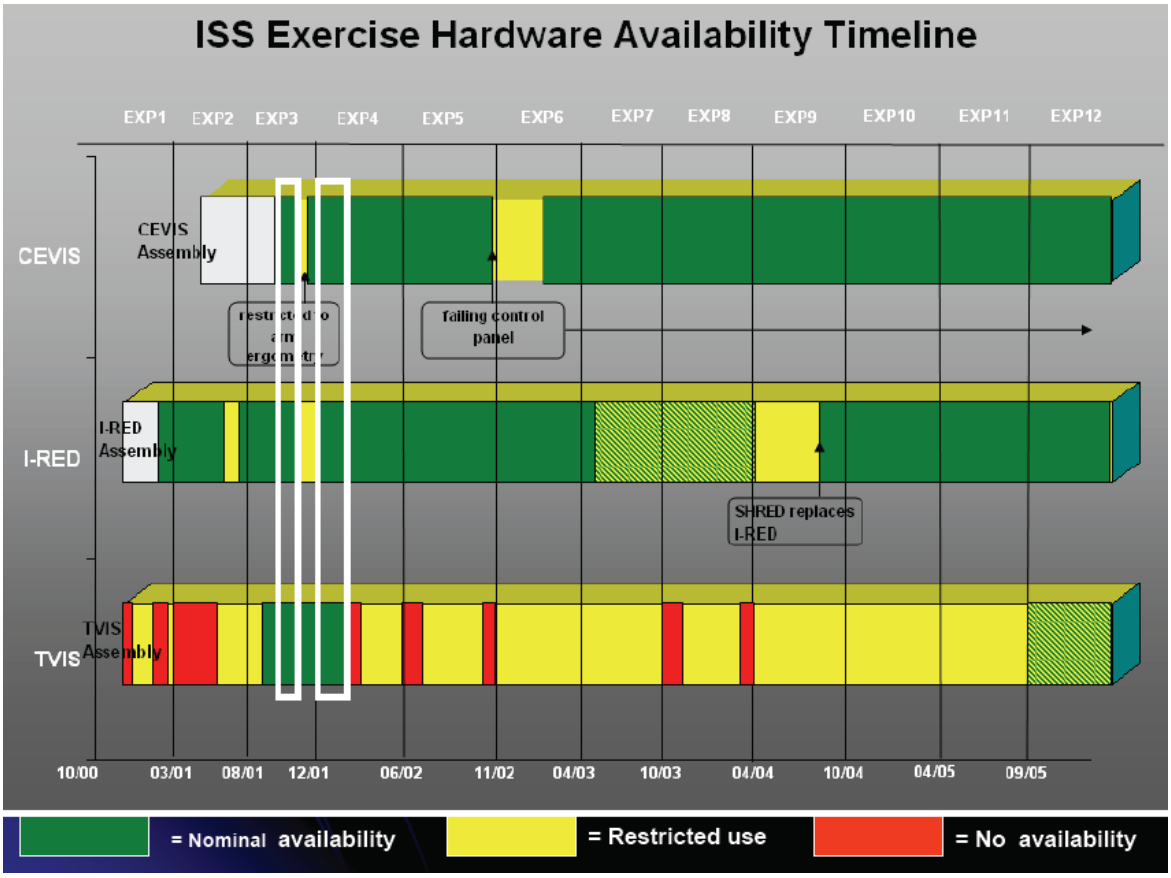

Figure 9. Exercise equipment failures and other constraints have limited the access of ISS crewmembers to the full complement of aerobic and resistance exercise protocols. Full capability for all 3 devices was present only for 2 short windows during Expeditions 3 and 4 (tall white rectangles).

Since the time depicted in Figure 9, both the reliability and capability of the ISS exercise countermeasures hardware have continued to mature. The second-generation treadmill (T2) and the Advanced Resistive Exercise Device (ARED, Figure 10) were delivered to ISS in 2009 and 2010, respectively. The T2 allows for motor-driven running speeds up to 15 mph in addition to being able to be used in a passive resistance mode (the user rather than a motor drives the belt against resistance). ARED provides adjustable loads of 600 pounds provided by vacuum canisters that provide a constant force and inertial flywheels that simulate the inertial loads that would be experienced using free weights in 1-G. ARED allows for most multi-joint bar-based resistance exercises to be performed, including the squat, deadlift, heel raise, and bench press. Additionally, ARED can support cable pull exercises with loads up to 150 pounds. ARED was delivered to the ISS with expectations of improving muscle outcome measures due to the additional load capacity and the changes in exercise prescription that this improvement affords.

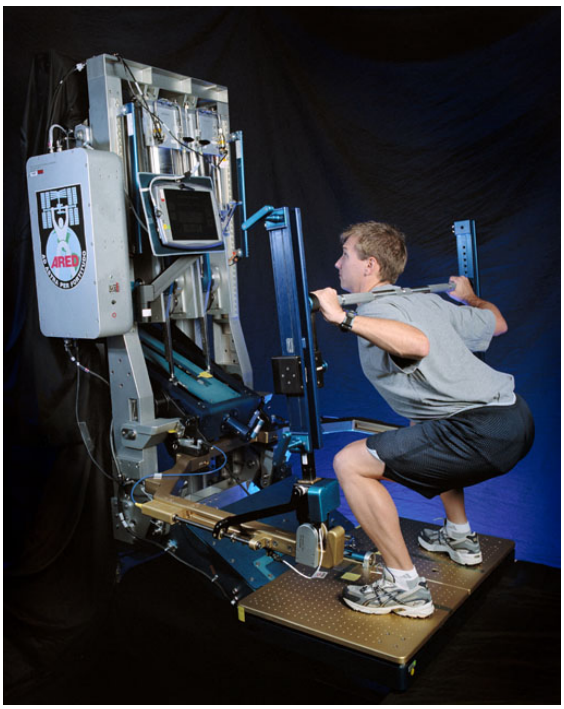

Figure 10. Ground version of the Advanced Resistive Exercise Device (ARED)

During ~6-month ISS missions, iRED crewmembers lost 0.42±0.39 kg of total body lean mass while ARED users gained 0.77±0.30 kg, as determined by whole-body DXA scans (Table 2); a limitation of these measurements is that the mean post-landing time required to obtain these measurements was 13±2 d and 8±1 d for iRED and ARED crewmembers, respectively. Regardless, a clear trend exists for the improved protection of muscle mass in more recent missions; this is likely due to a combination of enhanced resistance exercise loading (ARED) and improved caloric intake during flight (Smith 2012), two key factors in skeletal muscle outcomes during unloading. ARED users lost more fat mass during flight, but because of an increase in muscle mass, ARED crewmembers had a smaller net decrease in total body mass compared with iRED crewmembers.

Table 2. Post-flight changes in body composition (mean ± SE) in long-duration ISS crewmembers using iRED and ARED during their flight

	iRED	ARED
Lean mass (n=23, 23), kg	-0.42 ± 0.39	0.77 ± 0.30
Fat mass (n=23, 23), kg	-0.71 ± 0.38	-1.24 ± 0.31
Total body mass (n=23, 23), kg	-1.12 ± 0.49	-0.47 ± 0.43

All United States Operating Systems (USOS; NASA, Japan Aerospace Exploration Agency, European Space Agency, and Canadian Space Agency) crewmembers undergo specific medical requirement testing before and after their ISS missions. Part of this testing includes isokinetic muscle strength and endurance testing of the legs and trunk muscles. Post-flight testing occurs 5-

7 days after landing. In Figure 11, we present results as the percent change from pre-flight for isokinetic strength and endurance testing for crewmembers. Results are divided into two groups: those who used iRED and those who used ARED during their flight. Isokinetic strength around the knee joint was measured at 60°/s. For iRED crewmembers, the mean decrements in knee extensor and knee flexor strength were -13.7% and -19.5%, respectively. ARED users still exhibited losses of knee extensor and flexor strength, but the values were improved at -6.9 and -11.1, respectively. Muscle endurance was measured in knee extensor and flexor muscles based on total work production during a 20 repetition effort at 180°/s. The average loss for iRED crewmembers was -10.7% in knee extensors and -8.9% in knee flexors. Mean values for loss of endurance in ARED users were lower than that in iRED users (-7.5% for both knee extension and flexion), but any improvements were more subtle than those for strength. Studies in bed rest analogs for long-duration spaceflight deconditioning have typically shown that calf muscle mass and strength are more difficult to protect than quadriceps muscle mass and strength (Alkner and Tesch 2004b; Trappe et al. 2007b). Here, we show that calf muscle strength in ISS crewmembers using iRED was reduced 14.2% compared with pre-flight values. While this value is on par with knee extensor results (-13.7%), the improvement in ARED users' calf muscle strength loss is more modest (-11.6% versus preflight) than that of ARED users for the knee extensors. Trunk extensor strength losses equaled -7.4% and -5.5% for iRED and ARED, respectively.

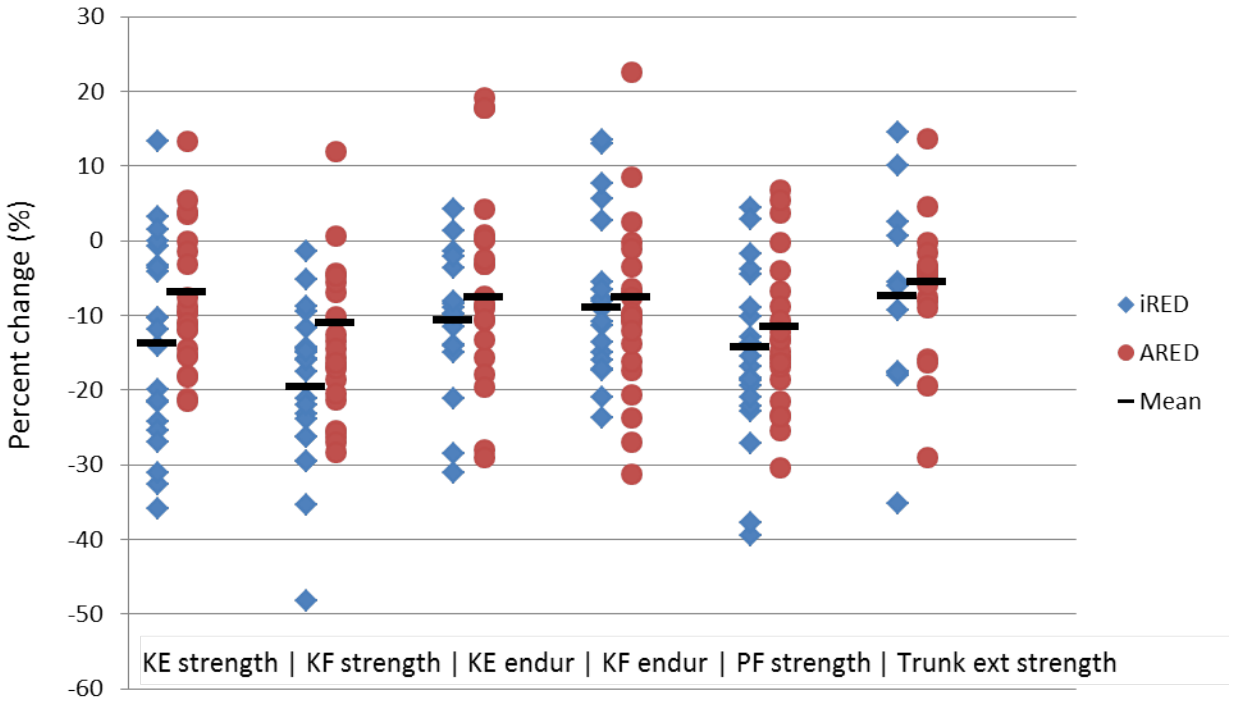

Figure 11. Post-flight changes (%) in isokinetic leg and trunk strength for long-duration ISS crewmembers using iRED and ARED during their flight.

The current permissible outcome limit for muscle strength in returning crewmembers is at or above 80% of baseline values (NASA Space Flight Human System Standard Volume 1: Crew Health; NASA-STD-3001). Although ARED-era crewmembers have fared better than iRED-era crewmembers, on average, both groups have losses of less than the 20% standard. However, an examination of the individual data shows that many individuals have lost more than the targeted

20% threshold. It is also important to keep in mind that the medical requirement testing is conducted approximately one week after landing and therefore may not reflect a crewmember's performance ability in the immediate post-landing time frame. While it would be ideal for all crewmembers to actually return with no loss of strength at all, it is important to note that this would not necessarily be reflective of crew ability to complete mission objectives. The Human Research Program aims to develop more performance-based strength standards that can better be used as benchmarks for mission success. Doing so will not only aid in designing better exercise countermeasure strategies, but will ultimately lead to greater assurance of crewmember safety.

While the delivery of ARED to the ISS already appears to be eliciting better strength maintenance, Human Research Program-funded research is beginning to examine how to better utilize ARED to not only improve strength and muscle mass outcomes, but also how to do so with a reduced weekly training volume. The Integrated Resistance and Aerobic Training Study (also known as SPRINT) is implementing a resistance training protocol based on greater intensity and reduced volume. Subjects are performing high load resistance training three days versus six days per week. In addition to pre- and post-flight muscle performance measures such as muscle mass, strength, power, and neuromuscular recruitment, in-flight measures of muscle mass will be tracked for the first time ever using ultrasound technology.

A recent investigation examined the effects of iRED versus ARED use onboard the ISS on body composition (Smith et al. 2012). Eight iRED users and five ARED users were subjected to DXA analysis prior to flight and again anywhere from 5 to 45 days post-flight (mean 12±11 days post-flight). Total body mass was unchanged in both groups; however, lean body mass was increased in ARED users and fat mass was reduced. These data are consistent with the view that ARED use is better for musculoskeletal outcomes following ISS missions; however, the effect of space flight on postural skeletal muscles following space flight is difficult to assess via whole-body lean mass, as the target tissues do not likely represent a large enough portion of the total lean mass pool to detect changes with sufficient accuracy. We attempted to determine whether the changes in muscle strength shown in Figure 11 correlated with pre- to post-flight changes in lean body mass and found that changes in strength correlated poorly with changes in total body lean mass. This result may be due to the aforementioned delays in obtaining these measurements post-flight or to the generic nature of total body lean mass changes as opposed to the greater specificity of leg lean mass or, optimally, regional changes in the quadriceps and calf muscle. It appears that MRI and potentially ultrasound imaging technologies are required to adequately detect morphological changes associated with loss of muscle strength.

Functional fitness test results for long-duration ISS crewmembers are presented in Table 3. Generally, iRED crewmembers experienced small to moderate decreases in performance of practical exercise tests such as pushups, pullups, bench press, and leg press. For all but one measure, ARED crewmembers fared better than their iRED counterparts, with most outcomes actually showing improvements after spaceflight.

Table 3. Post-flight changes (%) in functional fitness outcomes (mean ± SE) for long-duration ISS crewmembers using iRED and ARED during their flight

	iRED	ARED
Handgrip (n=10, 23)	-7.8 ± 2.7	-2.9 ± 1.3
Balance (n=10, 23)	0.0 ± 0.0	0.0 ± 0.0
Cone test time (n=10, 23)	15.9 ± 4.3	8.8 ± 2.0
Pushup (n=23, 22)	-11.7 ± 5.1	5.2 ± 3.9
Situp (n=22, 23)	-0.6 ± 3.7	4.0 ± 4.0
Pullup (n=19, 21)	-13.6 ± 5.9	13.5 ± 10.3
Sit and Reach (n=20, 23)	-9.5 ± 2.3	-8.1 ± 2.2
Bench Press (n=22, 22)	-4.4 ± 1.6	5.3 ± 1.9
Leg Press (n=20, 22)	-2.8 ± 1.5	-4.3 ± 1.8

Pre-flight tests were conducted approximately 70 d prior to launch; post-flight tests were conducted on R+8 ± 0.4 d, with no difference between iRED and ARED crewmembers.

Nutritional regulation of protein metabolism as it pertains to maintenance of muscle mass is a growing research topic with implications for aging populations and those undergoing unloading such as the ISS crew. Numerous investigations have addressed the roles of protein and amino acid intake in bed rest analogs for long-duration spaceflight (see below), whereas spaceflight data are much more limited. Aboard the ISS, protein intake has well-exceeded the U.S. Recommended Dietary Allowance (0.8 g/kg/d) both in the past (1.1 g/kg/d) and more recently (1.4 g/kg/d) (Smith et al. 2012). Total caloric intake has historically been a problem; Stein et al. reported significant decreases in body mass and protein synthesis after long-duration spaceflight on Mir. The reduction in protein synthesis was positively correlated with a decrease in energy intake during flight (r^2=0.86) (Stein et al. 1999). These findings demonstrate the synergistic, deleterious effect of reduced energy intake on skeletal muscle metabolism and mass during mechanical unloading. A more detailed discussion of these topics can be found in the Nutrition Evidence Report.

B. Human Ground-based Evidence

1. Models of Spaceflight Unloading

Several ground-based paradigms have been used to emulate the effects of microgravity unloading on human skeletal muscle, including complete horizontal or 6° head-down-tilt bed rest, dry immersion, and unilateral upper- and lower-limb unloading with or without joint immobilization. In general, skeletal muscle responses to unloading have been similar in all of these models. Although no perfect simulation of crew activities and the microgravity environment can be adequately achieved, Adams and colleagues have suggested that bed rest is an appropriate model of spaceflight for studying skeletal muscle physiological adaptations and

countermeasures (Adams et al. 2003). Absent from human analog studies are the unique operational and psychological stressors associated with spaceflight that exacerbate the physiological changes resulting from muscle unloading (Buckey 2006; Paddon-Jones et al. 2005). Finally, in anticipation of a future, long-duration human presence on the moon, Cavanagh et al. developed a lunar bed rest model that incorporates standing and sitting with axial skeleton loading of 1/6 body weight (Cavanagh et al. 2013).

2. Muscle Mass, Volume, and Strength

Bed rest unloading causes a significant loss of body nitrogen and lean body mass (Ferrando et al. 1996; LeBlanc et al. 1992; Stuart et al. 1990). A reduction in the size or volume of the ambulatory muscles accounts for most of the decrease in lean body mass after bed rest (Ferrando et al. 1995; Kortebein et al. 2007; LeBlanc et al. 1992; Paddon-Jones et al. 2004). Horizontal and 6° head-down-tilt bed rest protocols of durations ranging from 1-17 weeks have resulted in significant reductions in lower-limb muscle mass as measured by DXA (mass) or MRI (CSA or volume). Decreases in muscle volume after bed rest are paralleled by decreases in muscle strength and endurance (Table 4), as evidenced by significant reductions in angle-specific torque (Dudley 1989), isokinetic muscle strength (Dudley 1992; LeBlanc et al. 1992), and fatigability (Katkovskiy 1974). Similar losses in muscle volume and muscle strength and endurance have been observed after unilateral lower-limb suspension (Berg et al. 1991; Dudley 1992; Hather et al. 1992). Dry immersion, a whole-body-unloading paradigm with the added advantage of mimicking the reduced proprioceptive input encountered during spaceflight, also causes reductions in muscle volume, strength, endurance, electrical activity, and tone (Grigoryeva 1985; Koryak 2002; Kozlovskaya 1983; Kozlovskaya 1982; Miller et al. 2004; Netreba et al. 2004; Shenkman et al. 2004).

Decreases in muscle volume with unloading are rapid and persistent. LeBlanc et al. reported significant losses of ~6% in both the quadriceps and soleus/gastrocnemius of young men after 14 d of bed rest; although attenuated in rate, the losses in these muscles continued throughout 17 weeks of bed rest with final losses of 16-18% and 30% in the knee and ankle extensors, respectively (LeBlanc et al. 1992). Findings from short- (Alkner and Tesch 2004b; Berry et al. 1993; Dudley et al. 1989; Ferrando et al. 1996; Kawakami et al. 2001; Kortebein et al. 2007; LeBlanc et al. 1992; Paddon-Jones et al. 2004; Suzuki et al. 1996; Trappe et al. 2007b) and long-duration (Alkner and Tesch 2004b; LeBlanc et al. 1992; Mulder et al. 2009b; Trappe et al. 2007b) bed rest studies corroborate this pattern of rapid initial losses followed by reduced, but continued, decrements. Thus, although absolute decreases in muscle mass are greater with longer periods of unloading, rates of loss are higher in the first several weeks of disuse.

Young women appear to lose muscle volume at similar if not slightly faster rates as men during bed rest inactivity. In two separate investigations utilizing the same MRI methodology, young men and women both rapidly reduced quadriceps (-10% and -17%) and triceps surae (-16% and -18%) muscle volume after 29 d of bed rest (Alkner and Tesch 2004a; Trappe et al. 2007b). After 89 d (men) and 57 d (women) of bed rest in these same subjects, quadriceps (-18% and -21%) and triceps surae (-29% and -29%) muscle volumes were further reduced in both genders although at attenuated rates, particularly in the quadriceps in men. Thus, the gastrocnemius/soleus muscles are more vulnerable to unloading-induced losses than the quadriceps.

Strength decreases during unloading are 1.5-3 times that of muscle mass (percent change) (Alkner and Tesch 2004b; Kawakami et al. 2001; Kortebein et al. 2007; LeBlanc et al. 1992; Mulder et al. 2009b; Suzuki et al. 1996; Trappe et al. 2007b). Bamman and colleagues observed losses of 18, 17, and 13% in concentric, eccentric, and isometric plantar flexor peak torque, respectively, after 14 d of bed rest (Bamman et al. 1998), and Akima and his co-investigators observed a 16% decrease in knee extensor isometric torque after 20 days of bed rest (Akima et al. 2000). Although not specifically reported, subjects in an 89-d bed rest trial (Shackelford et al. 2004) experienced significant reductions in isokinetic torque in the lower body, with the greatest losses found in the knee extensors (-35%). This study also used isotonic testing (1RM), and mean losses ranging from -6 to -37% were observed; reductions in adductor, abductor, and leg press strength were on the order of ~25-30% (Shackelford et al. 2004). In an earlier 90-day bed rest trial, LeBlanc and colleagues observed losses of 31% in knee extension strength and 15% in knee flexion strength (LeBlanc et al. 1992). Similar to changes in muscle mass, unloading-induced strength losses are often greater in the ankle plantar flexors than in the knee extensors (Gogia et al. 1988; Mulder et al. 2009b; Trappe et al. 2007b), although this is not always the case (Alkner and Tesch 2004b; LeBlanc et al. 1992).

Few studies have reported changes in the ab/adductor or the flexor/extensor muscles of the hip. Shackelford et al. reported that isotonic strength decreased by approximately 25% in the adductors, but only a 6% decrease in the hip flexors was demonstrated after 17 weeks of bed rest (Shackelford et al. 2004). After 55 days of bed rest, Berg et al. reported that a 22% reduction in isometric hip extension occurred, although the extensor muscles in the gluteal region decreased in volume by only 2% (Berg et al. 2007). The authors reported no explanation for this discrepancy between the proportion of reduced strength relative to the loss of mass and also stated that no previous studies in the literature had made these concurrent strength/volume measurements in the hip musculature.

Table 4. Changes in leg lean mass (LLM), knee extension strength, and ankle extension strength in bed rest studies of various durations.

Study	BR duration (d)	Δ LLM, CSA, or volume (%)	Δ Knee ext strength (%)	Δ Ankle ext strength (%)
Alkner 2004	29	-10.0 (vol)		
	89	-18.0 (vol)	-60.0	
Bamman 1998	14		-14.5	
Berry 1993	30	-11.0 (CSA)		
Dudley 1989	30		-24	
Ferrando 1996	14	-3.9 (mass)		
Gogia 1988	35		-19.0	-24.4
Kawakami 2001	20	-7.8 (CSA)	-10.9	

Kortebein 2007	10	-6.3 (mass)	-15.6	
LeBlanc 1988	35			-26
LeBlanc 1992	7		-14.7	-7.2
	35		-25.2	-12.5
	119	-11.9 (vol)*	-30.7	-19.9
Mulder 2009	60	-13.5 (CSA)	-21.3	-24.9
Paddon-Jones 2004	28		-17.8	
Suzuki 1996	20	-10.6 (vol)	-23.6	
Trappe 2007	29	-16.8 (vol)		
	60	-21 (vol)	-33.7	-42.1

*Measured by dual-photon absorptiometry; vol = volume, CSA = cross-sectional area (Alkner and Tesch 2004b; Bamman et al. 1998; Berry et al. 1993; Dudley et al. 1989; Ferrando et al. 1996; Gogia et al. 1988; Kawakami et al. 2001; Kortebein et al. 2007; LeBlanc et al. 1988; LeBlanc et al. 1992; Mulder et al. 2009b; Paddon-Jones et al. 2004; Suzuki et al. 1996; Trappe et al. 2007b)

Decreases in strength are problematic due to the functional limitations that they impose. In a novel study utilizing a weighted suit to reduce subjects' relative strength, Ryder et al. determined strength and power thresholds below which functional task performance was impaired (Ryder et al. 2013).

3. Neural Influences

As enhanced neural function plays a significant role in the increased muscle strength associated with early adaptation to resistance exercise training (Moritani and deVries 1979), the reverse is also true, as decreases in neural function contribute to the reduction in strength observed with unloading. These neural maladaptations include decreased electrically evoked maximal force (Koryak 1995), reduced maximal integrated electromyography (Berg et al. 1997; Dudley 1992), increased submaximal electromyography (Berg et al. 1997), neuromuscular junction dysfunction (Grana et al. 1996), and reduced specific tension (Berg et al. 1997). After 23-d unilateral lower-limb suspension (ULLS), de Boer et al. observed an increased electromechanical delay and reduced rate of torque development during maximal voluntary contraction of the knee extensors, an effect also observed previously (Bamman 1998); however, the central activation ratio and normalized electromyography root mean square were unchanged (de Boer et al. 2007a). Fifty-six days of BR also caused no change in the normalized electromyography root mean square during maximal voluntary contraction of the knee extensors but elicited decreases in median firing frequency and fiber conduction velocity (Mulder et al.

2009a). Fiber conduction velocity of the vastus lateralis and tibialis anterior was also reduced in both single motor units and whole muscle during submaximal contractions after 14 d of bed rest (Cescon and Gazzoni 2010).

4. Muscle Protein Synthesis, Breakdown, and Cell Signaling

The primary mechanism of muscle loss during unloading is a reduction in muscle protein synthesis (MPS) (de Boer et al. 2007b; Glover et al. 2008; Kortebein et al. 2007; Paddon-Jones et al. 2006; Symons et al. 2009). This reduction is significantly correlated with the decrease in muscle mass during bed rest (Ferrando et al. 1996; Ferrando et al. 1997). Activation of the mammalian target of rapamycin (mTOR) pathway, a key regulator of translation initiation, is required to stimulate muscle protein synthesis (Dickinson et al. 2011). It is thus not surprising that bed rest causes a diminished phosphorylative response of mTOR and its downstream targets, ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1), to anabolic stimuli (e.g., essential amino acids) (Drummond et al. 2012).

Muscle protein breakdown (MPB) is more difficult to measure, and several studies have shown no changes with bed rest (Ferrando et al. 1996; Stuart et al. 1990; Symons et al. 2009); however, recent evidence suggests that MPB plays an important role in the atrophic response to unloading, particularly during the first days of disuse. Tesch et al. used a microdialysis technique to sample the 3-methylhistidine concentration in the vastus lateralis before and after ULLS and found a 44% increase in muscle proteolysis after only 72 h of unloading (Tesch et al. 2008). In their brief review, Attaix et al. noted a number of recent studies that demonstrate a strong interconnectedness between the regulation of synthetic and proteolytic pathways during disuse; however, none of this work was performed in humans (Attaix et al. 2012). Marimuthu et al. argue that, in light of observed increases in ubiquitin-protein conjugates (Glover et al. 2010) and 3-methylhistidine (Tesch et al. 2008), an early and transient increase in muscle protein breakdown is partially responsible for disuse-induced muscle atrophy (Marimuthu et al. 2011).

5. Fiber Changes and Enzyme Activity

At the structural level, the loss of muscle volume in disuse models correlates with a significant decrease in the CSA of both Type I and Type II myofibers (Bamman et al. 1998; Berg 1997; Hather et al. 1992; Hikida et al. 1989; Hortobagyi et al. 2000; Il'ina-Kakuyeva 1979; Rudnick et al. 2004). In general, Type II myofibers seem to be more likely to atrophy than Type I myofibers during short-term unloading, with no significant myofiber type shifting observed (Bamman et al. 1998; Bamman et al. 1997; Berg 1997), although alterations in total muscle MHC protein isoform expression have been reported (Bamman et al. 1999). However, in prolonged, 84-d BR, Type I fibers in the vastus lateralis atrophied to a greater degree (-15%) than Type IIa fibers (-8%), and a shift to a faster fiber type occurred in both the vastus lateralis (Type I to Type IIa and Type IIa to Type IIx) and the soleus (increased hybrid fibers) at the expense of Type I fibers (Gallagher et al. 2005; Trappe et al. 2004). More recently, 35-d BR caused large, but relatively uniform, CSA decreases of 31%, 21%, and 28% in Type I, Type IIa, and Type IIx vastus lateralis myofibers, respectively (Brocca et al. 2012).

Immobilization by limb casting does not seem to reduce the relative proportions of muscle-specific proteins, such as carbonic anhydrase II and myoglobin, over that predicted by the overall decrease in muscle protein synthesis (Virtanen et al. 1991). In contrast, experimental evidence

suggests that the specific activity of muscle enzymes involved in oxidative metabolism, such as pyruvate dehydrogenase, is decreased by cast immobilization (Ward et al. 1986). A similar reduction in the activity of citrate synthase, but not phosphofructokinase, has been detected in the vastus lateralis, indicating a significant impairment of the oxidative capacity in this muscle after unilateral limb suspension (Berg et al. 1993). The differences observed between cast immobilization and unilateral limb suspension or bed rest protocols may indicate that the former is a better model of muscle atrophy induced by hypokinesia and that the latter two are better models of muscle atrophy induced by muscle hypodynamia. The latter situation more closely resembles the actual conditions experienced by crewmembers during spaceflight, namely, removal of mechanical loading without a reduction in limb mobility.

6. Insulin Resistance

Additional research findings exist that relate peripherally to this risk description that should remain associated with it. First, secondary to the decrease in muscle mass associated with mechanical unloading is an increased susceptibility to insulin resistance and glucose intolerance. Second, crewmembers chronically exposed to the microgravity environment may develop impaired body temperature regulation during rest and exercise that may lead to heat strain and injury. These effects are discussed more fully in the paragraphs that follow.

Bed rest studies (Dolkas and Greenleaf 1977; Lipman et al. 1972) have shown an increased insulin response to glucose tolerance tests. Plasma insulin levels have increased up to four-fold compared with those of control subjects, and blood glucose levels exceeded those of the controls 2 h after glucose loading. Similarly, Stuart et al. reported impaired glucose tolerance and a greater than 40% increase in both fasting plasma insulin and the insulin response to a glucose challenge. Suppression of hepatic glucose production by insulin was unchanged after bed rest, indicating that insulin sensitivity was reduced only in skeletal muscle and not in the liver (1988). After 28 d of bed rest, Brooks et al. showed an increase in fasting insulin levels in an amino acid-supplemented group, whereas amino acid supplementation with resistance exercise decreased insulin values during bed rest; these changes were negatively correlated with changes in mid-thigh muscle area and were positively associated with whole body fat mass (Brooks 2008).

7. Heat Stress and Thermoregulation

Human expenditure of energy results in the generation of heat. The body heat generated by normal activities, and particularly by exercise, triggers homeostatic regulatory mechanisms with the goal of maintaining body core temperature within its relatively narrow, safe physiological range by means of vasoregulation and diaphoresis. The weightless environment of spaceflight may impair heat dissipation by reducing evaporative and conductive heat exchange. Microgravity and spaceflight may perturb the body's thermoregulatory mechanisms by altering the work efficiency, metabolic rate, or circadian rhythms of heat production. Additionally, human space travelers are often not well hydrated, have a 10-15% decrease in intravascular fluid (plasma) volume, and may lose both their preflight muscular and cardiovascular fitness levels as well as their thermoregulatory capabilities. As a result, they may become less heat-acclimated or may acquire an altered thermal sensitivity (Fortney 1991).

Alterations in thermoregulation in association with spaceflight could have significant impacts on a variety of spaceflight-associated activities, including exercise as a countermeasure

to muscle atrophy, cardiac deconditioning, and bone loss; EVA; and vehicle landing and egress. EVA suits and launch and entry or advanced crew escape suits (ACES) worn by ISS and Shuttle crewmembers are designed to provide an impermeable barrier between the wearer and the external environment. To compensate for the lack of heat exchange through the fabrics of these suits, the EVA suit provides both liquid (conductive) and air (convective) cooling, while a liquid cooling garment is worn under the ACES in addition to a hose connection to forced orbiter cabin air. Thus, crewmembers with altered thermoregulatory capabilities are at even greater risk if failure of the cooling systems of these garments occurs (Pisacane et al. 2007). Manifestations of altered thermoregulation include increased heart rate and body temperature during exercise, decreased work capacity and endurance, decreased post-flight orthostatic tolerance, decreased cognitive ability, and a delay in recovery of exercise capacity and endurance after flight (Fortney et al. 1998).

Thermoregulation has been studied in association with both spaceflight (Fortney et al. 1998; Greenleaf 1989) and 6˚ head-down-tilt bed rest (Greenleaf 1989; Greenleaf and Reese 1980; Lee et al. 2002). To date, there have been no direct measurements of heat balance during in-flight exercise sessions. In the only spaceflight study, submaximal exercise and thermoregulatory responses were recorded before flight and at 5 d after landing in two crewmembers who completed a 115-d mission (Fortney et al. 1998). Normal heart rates were observed for both crewmembers during supine exercise for 20 min each at 20% and 65% of VO_{2max}. However, during post-flight (five days after landing) testing, exercise was voluntarily discontinued after only 8-9 min of supine exercise at the 65% of VO_{2max} level for the two crewmembers when they both experienced difficulty in maintaining pedaling frequency and complained of leg fatigue, and their heart rates exceeded the highest recorded pre-flight levels. Both crewmembers exhibited a more rapid increase in body core temperature during the shorter post-flight exercise session than during the pre-flight session; it was concluded that heat production was not altered but that impairment of heat dissipation due to altered vasodilatory and sweating responses were responsible for the increased rate of rise in the core body temperature.

8. Nutrition

Reduced energy intake during unloading greatly exacerbates lean tissue loss. Biolo et al. studied young men during 14 d of bed rest with either eucaloric energy intake or a 20% hypocaloric diet similar to the energy deficits reported in spaceflight (Biolo et al. 2007). During eucaloric bed rest, subjects lost a small amount of lean mass (300 g), while hypocaloric bed rest provoked an almost four-fold greater decrease (1100 g). This magnified loss in the hypocaloric condition was facilitated by greater whole body net protein catabolism (i.e., MPS < MPB) in the post-absorptive state (Biolo et al. 2007). Excess energy intake during bed rest unloading is also deleterious and accelerates lean mass loss via increased systemic inflammation (Biolo et al. 2008). Thus, it appears that targeted eucaloric intake is key to the maintenance of lean mass during mechanical unloading.

Protein intake also plays a key role in the protection of lean mass during disuse. A simple 7-d bed rest study demonstrated that low protein intake (0.6 g/kg/d) causes a reduction in whole body protein synthesis while higher intakes (1.0 g/kg/d) during bed rest prevent this (Stuart et al. 1990). Nutrition during unloading is further discussed below in the Countermeasures section (Section 10).

9. Aging Effects

Age also modulates unloading-induced muscle loss. Although muscle loss in young adults (30-35 y) during inactivity is considerable (e.g., 300-600 g leg lean mass in 14 d) (Biolo et al. 2007; Ferrando et al. 1996), older adults (67 y) lose lean mass at more than double the rate (950 g leg lean mass in 10 d) of their younger counterparts (Kortebein et al. 2007); not surprisingly, these reductions are accompanied by significant decrements in muscle strength and power (Kortebein et al. 2008). Similar to young adults, bed rest-induced muscle atrophy in older adults is mechanistically driven by a reduction in post-prandial muscle protein synthesis, mTOR signaling, and amino acid transporter content (Drummond et al. 2012). No published data exist for unloading-induced alterations in muscle mass and metabolism in middle-aged individuals who would be representative of typical crewmember age. However, combined with the insidious onset of sarcopenia around the age of 40 (English and Paddon-Jones 2010), it is likely that middle-age (40-55 y, astronaut age) is associated with an accelerated rate of inactivity-induced alterations in muscle compared with young adults.

10. Countermeasures

Exercise and nutrition are the primary interventions that have been employed in ground-based models to prevent unloading-induced changes in skeletal muscle. Exercise countermeasures are largely effective in preventing deleterious changes in skeletal muscle during unloading, while nutritional interventions are only somewhat, if at all, protective, particularly when employed in the absence of exercise. Because the scope of this risk is the impact of changes in muscle mass, strength, and endurance, we will focus on resistance exercise countermeasures and will examine aerobic countermeasures only when they were employed in conjunction with resistance exercise.

In short-duration bed rest, resistance exercise (80-85% 1-RM) preserved muscle strength of the thigh and calf (Bamman et al. 1998; Bamman et al. 1997). Protection of muscle volume occurred through the maintenance of protein synthesis, which also likely influenced muscle strength (Ferrando et al. 1997). Similarly, Akima et al. were able to maintain isometric peak torque in subjects who performed daily maximal isometric contractions of the knee extensors during 20 d of bed rest (Akima et al. 2000). In long-duration bed rest (119 d), Shackelford et al. preserved isokinetic muscle strength and observed substantial increases in isotonic muscle strength using an aggressive resistance exercise training protocol (Shackelford et al. 2004). During 90 d of bed rest, a flywheel resistance exercise device capable of providing eccentric overload (i.e., loading that is greater during the descent phase of a lift than during the ascent phase) prevented the loss of muscle mass and strength in the thigh and attenuated losses in the calf (-15% muscle volume vs. -29% in controls) and in peak power and displacement during a vertical jump (Alkner and Tesch 2004b; Rittweger et al. 2007). This differential response of the quadriceps (knee extensors) and gastrocnemius/soleus (plantar flexors) to exercise countermeasures during unloading is a common finding. Using the same exercise device and resistance exercise protocol but with the addition of aerobic treadmill running (a vertical treadmill with lower body negative pressure, LBNP), Trappe et al. replicated their previous results in the thigh (protection of both muscle mass and strength) and improved calf outcomes (-8% muscle volume and maintenance of strength) during 60 d of bed rest in women compared

with their resistance exercise-only countermeasure (Trappe et al. 2007b). The preservation of muscle mass and function in the thigh was facilitated by maintenance of MHC I and IIa single fiber size and function (Trappe et al. 2007a). A recent bed rest study demonstrated that a combined, high-intensity resistance and aerobic exercise program could fully protect aerobic capacity, leg press power, and quadriceps CSA over 14 d of unloading (Ploutz-Snyder et al. 2013).

Other resistance training modalities employed during bed rest include centrifugation (artificial gravity), vibration, and neuromuscular electrical stimulation. A 21-d bed rest study with 1 h/d centrifugation (2.5 G_z at the feet) attenuated decrements in both knee extensor and plantar flexor torque-velocity relationships and muscle fiber CSA in both the vastus lateralis and soleus; artificial gravity was unable to maintain total MHC mRNA content or the slow to fast fiber type conversion in the soleus (Caiozzo et al. 2009). Mechanistically, post-absorptive muscle protein synthesis was maintained in the vastus lateralis and soleus with centrifugation in contrast to controls, who exhibited a 49% reduction in vastus lateralis muscle protein synthesis and a non-significant 22% decrease in the soleus; muscle protein breakdown was unchanged in both groups (Symons et al. 2009). Resistance exercise prevented (thigh) or attenuated (calf) decreases in muscle CSA and isometric peak torque during 60 d of bed rest, but the addition of vibration to the resistance exercise protocol did not improve muscle outcomes (Mulder et al. 2009b), and whole body vibration alone was ineffective in preventing decreases in leg volume during even a brief 14-d bed rest period (Zange et al. 2009). However, in combination with resistance exercise, vibration during long-duration bed rest did show efficacy in preventing negative changes in bone (Belavy et al. 2011). Neuromuscular electrical stimulation was effective in preventing both a decrease in quadriceps CSA and increases in mRNA of several negative muscle regulators, but it was unable to protect muscle strength during a brief 5-d period of unilateral limb suspension (Dirks et al. 2014).

Several studies have examined the effectiveness of supplemental protein or essential amino acids as a countermeasure to unloading-induced adaptations in skeletal muscle; some have shown positive effects (e.g., attenuated lean mass loss) (Ferrando et al. 2010; Paddon-Jones et al. 2004; Stuart et al. 1990), while others have not (Brooks et al. 2008; Stein et al. 2003; Trappe et al. 2007b). In light of suggestions that the Recommended Daily Allowance (RDA) of 0.8 g protein/kg/d is too low (Layman 2009; Paddon-Jones and Rasmussen 2009; Wolfe et al. 2008), Stein and Blanc have argued that the positive outcomes of supplemental protein or essential amino acids in bed rest are simply due to the provision of adequate total protein (e.g., 0.8 g/kg/d in control diet + 0.6 g/kg/d supplementation = 1.4 g/kg/d) and not to an effect of the supplement *per se* (Stein and Blanc 2011); this view is supported by the fact that the studies with negative findings for supplemental protein/essential amino acids all provided a control diet of ≥ 1.0 g protein/kg/d. Despite this seemingly simple conclusion to the supplemental protein/essential amino acids question, other research suggests that provision of adequate, or ideally, optimal, protein intake is more complex than just g/kg/d and is also modified by age (Wolfe et al. 2008). In a 7-d study of ambulatory young adults, Mamerow et al. examined the effects of protein distribution across the three daily meals on muscle protein synthesis; because of their young age and ambulatory status, these subjects could be assumed to be the least responsive to this type of subtle intervention. Regardless, the provision of protein in an evenly distributed pattern of 30 g/meal (90 g protein/d) elicited greater muscle protein synthesis than consuming the same 90 g/d in a 10/15/65 g split across breakfast, lunch, and dinner (Mamerow et al. 2013). This practical intervention is based on mechanistic work that shows that muscle protein synthesis is maximally

stimulated by 10-15 g of essential amino acids (~30 g whole protein) and, perhaps more importantly, by ≥ 3 g leucine (Churchward-Venne et al. 2012; Devkota and Layman 2010; Wall et al. 2013a). The influence of age on the acute, meal-based muscle protein synthetic response is seen in the work by Katsanos et al., which showed that in contrast to young adults, older adults had an attenuated muscle protein synthetic response to a small serving of essential amino acids that contained only 1.7 g leucine but that the response was normalized to that of the young controls with the addition of 1.1 g leucine (2.8 g leucine total) (Katsanos et al. 2005; Katsanos et al. 2006). Similarly, Rieu et al. demonstrated that a mixed meal with 30 g protein but only modest leucine content (2.4 g leucine) when supplemented with additional leucine (~3.9 g; total leucine = 6.3 g), increased muscle protein synthesis in older adults while the same meal with supplementary alanine failed to do so (Rieu et al. 2006). Together, these data underscore the importance of adequate essential amino acid/leucine intake at each meal to maximally stimulate muscle protein synthesis, particularly in older adults. Given that these studies demonstrated an anabolic resistance in older, ambulatory individuals, it is likely that careful, meal-based protein/leucine intake is necessary to optimize skeletal muscle outcomes during unloading when even young adults quickly become resistant to the anabolic effects of essential amino acids (Glover et al. 2008; Wall et al. 2013b).

Only one study has evaluated the combined effects of resistance exercise and nutrition countermeasures during unloading. Brooks et al. showed that a 15 g essential amino acid supplement (with 2.8 g leucine) provided either before or after daily resistance exercise training during 28 d of bed rest was largely able to protect skeletal muscle mass and function while the supplement without exercise was significantly less effective; unfortunately, the study did not include a resistance training-only control group to facilitate an understanding of any additive effect that the nutritional supplement may have provided (Brooks et al. 2008). A final piece of ground-based evidence salient to the optimization of skeletal muscle outcomes via nutrition involves the provision of supplemental protein immediately prior to sleep. Res et al. demonstrated that 40 g of protein ingested just before bedtime was effectively digested and absorbed, increased muscle protein synthesis, and improved net balance overnight (Res et al. 2012).

In summary, resistance exercise is an effective countermeasure to ground-based, unloading-induced alterations in skeletal muscle although total protection of the calf muscles has proven somewhat elusive; high intensity (i.e., % concentric 1-RM) and eccentric loading equivalent to or greater than concentric loading (i.e., eccentric overload) appear to be key modifiers of exercise efficacy. Artificial gravity via centrifugation is a promising intervention for skeletal muscle during unloading, the efficacy of which will likely be improved with the addition of dynamic, resistance exercise movements during centrifugation. Whole-body vibration is an ineffective countermeasure for muscle during unloading and does not improve the efficacy of resistance exercise regimens, although positive effects on bone have been reported when combined with resistance exercise. Nutritional inadequacies (e.g., insufficient protein or energy intake) can exacerbate muscle losses during unloading, while protein/essential amino acid supplementation affords only partial or, in some cases, no protection for muscle when employed alone. However, efforts to optimize the anabolic potential of dietary protein within the context of adequate total energy and protein intake should include further work examining the effects of protein distribution across meals and of supplementation prior to prolonged periods of fasting such as before sleep; as a potent anabolic agent, the essential amino acid leucine should be a primary candidate for supplementation. Little or no evidence exists to describe the synergistic effects of

supplemented/optimized protein intake and exercise countermeasures during unloading, nor, conversely, does work that elucidates the impact of sub-optimal nutrition on otherwise effective exercise countermeasures.

11. Summary

Some general conclusions that can be drawn from the above ground-based human studies are as follows: first, terrestrial unloading models produce selective atrophy in the muscles of the lower limbs, especially the anti-gravity muscles; second, this response is greater in the extensor muscles than in the flexor muscles; third, muscle atrophy occurs quickly (within 7–14 days) in response to unloading; fourth, loss of muscle mass is paralleled by decrements in muscle strength and endurance, but strength losses are greater than volume losses; fifth, long-duration terrestrial unloading produces a slow-to-fast shift in absolute myofiber characteristics and alters the expression of MHC isoforms in human muscle so that an increase in MHC hybrid myofibers is observed, resulting in a faster phenotype; sixth, high-intensity resistance exercise (ideally coupled with aerobic exercise) is highly (quadriceps) to moderately (calf) protective of muscle mass and strength; and seventh, other countermeasures have demonstrated efficacies ranging from promising (centrifugation), to partial (nutrition), to poor (vibration).

C. Summary of Experimental Animal Studies

The goal of this section is to provide insight into animal research pertinent to the risk of impaired performance due to reduced muscle mass, strength, and endurance of the skeletal muscle system. This section will focus on two primary themes. Theme I will address the historical information that has been accumulated from spaceflight studies and ground-based analogues of skeletal muscle unloading such as the hindlimb suspension (HS) model. Theme II will address recent studies from 2008 to the present concerning the mechanisms impacting skeletal muscle atrophy along with exercise and molecular strategies designed to ameliorate muscle wasting. In the context of this presentation, the authors call attention to three key publications that provide important information impacting animal research relevant to the Human Research Program. The first involves the recent Decadal Study Report on "Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era", which was published by the Space Studies Board of the National Academies in 2011.

In addition, the authors point out two recent review articles relevant to skeletal muscle homeostasis and muscle wasting by Baldwin et al. (Baldwin et al. 2013) and Brooks and Myburgh (Brooks and Myburgh 2014)**.** Although it is beyond the current theme of homeostasis in skeletal muscle, the authors encourage the readers to examine the exciting recent findings of Michael Delp and colleagues concerning mechanisms and functional consequences of vascular remodeling in skeletal muscle by Stabley et al. (Stabley et al. 2013) and Sindler et al. (Sindler et al. 2009).

1. Theme I: Historical Research Involving Spaceflight Studies and Ground-Based Analogs of Unloading

This section summarizes the studies that have been conducted on animal subjects (such as rodents and non-human primates) that have been exposed either to spaceflight or (in the case of rodents) to the well-accepted ground-based analog of HS to ascertain the effects of unloading states on the properties of muscle mass, strength, and endurance. The results presented herein overwhelmingly corroborate the body of evidence that has been reported on human subjects in the preceding sections of this report. Importantly, through the use of more cellular and molecular analyses, greater insights have been obtained into the underlying mechanisms associated with these alterations in muscle structure and function. Because the majority of evidence concerning the effects of spaceflight on mammalian skeletal muscle has been derived from rodent studies, the information provided here is focused primarily on the rodent model. It is important to point out that the structure and function of rodent skeletal muscle are nearly identical to those of human skeletal muscle. For example, rodent muscle is composed of the same general fiber-type profile and is sensitive to the same environmental (mechanical, hormonal, metabolic) cues observed in human muscle. Thus, the information summarized below provides credence to the data base derived from human subjects. However, it is important to point out that one primary advantage of the rodent model is that adaptive changes that occur in both species unfold in a much shorter time frame in rodents than in humans (hours to days versus days to weeks), making it possible to predict long-term changes in human skeletal muscle based on the shorter absolute time frame of the studies performed in rodents. Another important consideration in the context of animal research during spaceflight is that one can perform a straightforward experiment in which there is no requirement to provide some type of countermeasure intervention as there is for humans, thereby avoiding the introduction of a confounding variable in ascertaining the true effects of spaceflight on a wide range of physiological variables. Additionally, given the remarkable agreement in the quantitative and qualitative nature of the findings observed in the spaceflight studies versus those obtained from ground-based HS studies, we have chosen to combine and integrate significant portions of the data that have been gathered in the last 25 years. This rodent database in space life sciences research includes 14 flight experiments, with 8 sponsored by the Russian Cosmos Program and 6 sponsored by NASA Space Life Sciences (SLS) and Space Transportation System (STS) missions (Thomason and Booth 1990; Thomason et al. 1987; Thomason et al. 1992). These flight experiments are complemented by numerous ground-based research studies that focused collectively on the topics described below. Most importantly, all of the data reported in this summary are derived from animal cohorts in which the control animals were studied from a synchronous vivarium group of the same age, strain, and gender, and the analyses were performed at the same time as that of the experimental groups. The provided information is based entirely on peer-reviewed experiments as detailed in the bibliography provided.

a) Activity Patterns of Rodents during Spaceflight

While recorded observations during spaceflight are less extensive in rodents (due to fewer flight missions with opportunities for astronauts or payload specialists to observe them), the available data suggest that rodents rely less on the hindlimbs for executing most movement

patterns (as is the case for humans). During spaceflight, their ankles appear to assume a plantar flexed position that may reduce the passive tension (force) imposed on the triceps surae group, of which the antigravity slow-twitch soleus muscle is a chief component (Recktenwald et al. 1999). A similar posture has been observed in the ground-based analog of HS. This posture is thought to affect the residual tension placed on this muscle group in the absence of a normal weight-bearing state, i.e., the ankle plantar flexor muscle group becomes truly unloaded. While electromyographic studies on adult rodents have not been conducted during spaceflight, studies performed on rodents during chronic HS indicate that only a transient reduction occurs in the electrical activity of the ankle plantar flexor muscles (soleus and medial gastrocnemius) (Alford et al. 1987). This pattern of activity is consistent with the posture of the muscle and the maintenance of muscle mass during the 28-day time frame of the experiment, i.e., the EMG activity was well maintained, while the ongoing atrophy was maintained. These findings reinforce the notion that it is the mechanical activity rather than the electrical activity imposed on the muscle that is essential to maintaining physiological homeostasis of muscle mass.

b) Observations of Activity Patterns during Early Recovery from Spaceflight

When animals return from spaceflight of even a short duration (days), their basic activity patterns are altered. The center of gravity in rats is much lower than normal. They no longer support their body weight and initiate movement off the balls of their feet, and the ankle joint assumes an exaggerated dorsiflexed position (Recktenwald et al. 1999; Riley et al. 1996). Movement for most voluntary activities is much slower and more deliberate (the animals cover smaller distances per unit time), and the animals spend significantly less time in bipedal stances (Recktenwald et al. 1999; Riley et al. 1996). Furthermore, the rodents use their tails for basic support to a greater degree, based on observations by the investigators. Thus, rodent motor skills and basic locomotor capability have less fidelity and capacity during posture maintenance and locomotion during the early stages of recovery; however, by 9 days after flight, the activity properties return to those seen in normal conditions.

c) Effects of Spaceflight and Hindlimb Suspension on Muscle Mass, Protein Content, and Gross Morphological Properties of Skeletal Muscle

Considerable information has accumulated covering a large number of spaceflight and HS experiments that span a time frame of ~4 to 22 days for spaceflight and from 1 to 56 days for HS. These experiments have primarily focused on extensor muscles used extensively for postural support and locomotor activity. The review by Roy, Baldwin, and Edgerton provides one of the most comprehensive reviews on rodents in the space environment (Roy et al. 1996a)**.** Additional reviews on this topic have been published (Baldwin 1996a; Baldwin 1996b; Baldwin et al. 1990; Baldwin et al. 1993; Dupont et al. 2011; McDonald et al. 1992b; McDonald and Fitts 1993). The collective observations clearly show that these types of muscle undergo significant reductions in muscle mass (muscle weight) (Caiozzo et al. 1994; Caiozzo et al. 1996; Ilyina-Kakueva et al. 1976; Martin et al. 1988; McDonald et al. 1992b; McDonald and Fitts 1993; Ohira et al. 1992)

along with a concomitant loss in total protein and myofibrillar (the fraction that is composed of the contractile machinery of structural proteins) protein content of the targeted muscles (Baldwin et al. 1990; Fitts et al. 1986; Roy et al. 1991; Roy et al. 1996a). In some experiments, it has been reported that the myofibrillar fraction can be degraded to a greater extent than other muscle fractions (Baldwin et al. 1990). The general pattern demonstrates that a rapid loss in muscle weight and net total and myofibrillar protein content (concentration (mg/g X muscle weight)) occurs during the first 7-10 days of unloading, which is followed by a more gradual loss in these constituents (Thomason and Booth 1990; Thomason et al. 1992). The net result is that between 25 and 46% of the muscle mass can be lost in antigravity muscles of the lower extremity, such as the soleus (Sol; a calf muscle) and vastus intermedius (VI; a deep layered quadriceps muscle), which are composed mostly of the slow Type I myofibers containing the slow MHC protein. MHC is the most abundant protein expressed in striated muscle, and this structural / regulatory protein serves as the motor protein that regulates, in synergy with its companion protein actin, the contraction process that derives the force, work, and power generation that is necessary for the muscle groups to bring about both movement and stabilizing types of activity (posture). It is also important to point out that fast-twitch synergistic muscles (expressing fast isoforms of MHC) are also targeted, but these muscles and their fibers are apparently not as sensitive to the unloading stimulus as the slower types of muscle. Compared with both the slow and fast types of muscle, atrophy of the corresponding joint flexors, such as the tibialis anterior and extensor digitorum longus muscles in the leg, is markedly lower (Roy et al. 1996a). Histochemical and immunohistochemical analyses at the single-fiber level clearly show that the atrophic process seen at the gross level is due to a reduction in the diameter of the affected myofibers of which the individual muscles are composed. These observations show that the slow fiber type is more sensitive than the faster fiber types, which is consistent with the gross muscle mass assessments (Gardetto et al. 1989; Haddad et al. 1993; Musacchia et al. 1992; Ohira et al. 1992). As a rule, regardless of the muscle, the larger fibers, whether fast or slow, are more sensitive to the unloading stimulus than their smaller counterparts (Roy et al. 1996a)**.**

d) Muscle Fiber Phenotype Remodeling in Response to Spaceflight and Hindlimb Suspension

Accompanying the atrophy process noted above are the important observations that many (but not all) of the slow fibers in primarily antigravity-type muscles (e.g., SOL and VI) are also induced to express fast myosin isoforms (Caiozzo et al. 1994; Caiozzo et al. 1996; Diffee et al. 1991; Haddad et al. 1993; Thomason et al. 1992). This transformation is largely manifested in the expression of hybrid fibers, in which both slow MHC and either fast type IIx or fast type IIa MHC become simultaneously co-expressed (Caiozzo et al. 1994; Musacchia et al. 1992). These observations suggest that the slow MHC is targeted for degradation, evidenced by the net loss in slow MHC in the atrophying muscle (fibers) (Baldwin et al. 1990; Thomason et al. 1992), while at the same time, according to pre-mRNA and mRNA analyses, up-regulation of the faster MHC genes by transcriptional and/or pretranslational processes occurs (Giger et al. 2009; Haddad et al. 1993; Pandorf et al. 2006; Van Gammeren et al. 2009). More recent studies on this topic clearly suggest that the type IIx MHC, which is a faster isoform than the IIa type, is more abundantly expressed. From these observations, it is apparent that the myofibrillar fraction, which is a key component of the muscle, is targeted for net degradation (as noted above) for two reasons: [1]

degradation of this fraction allows smaller-diameter fibers to meet the reduced requirements for force generation, and [2] the unraveling of the myofibrillar system allows faster MHC isoforms to become incorporated into the contractile machinery to replace the slower isoforms so that the muscle is able to function more effectively under a reduced state of gravitational loading. Providing further insight is the observation that the unloading state of spaceflight and of HS also increases the expression of fast type II sarcoplasmic reticulum (SR) ATPase-driven calcium pumps (SERCA II) while repressing the slower type I SERCA calcium pump (Schulte et al. 1993). Because calcium cycling is used to regulate fiber activation and relaxation, the SR component of the muscle fiber controls the synchrony of contraction-relaxation processes. Because calcium cycling and cross bridge cycling are the two major systems that account for the vast majority of the energy expended during muscle contraction to support movement, when this property of the muscle is switched to a faster system, the muscle can function more effectively in the unloaded environment. However, when the muscle encounters environments with a high gravitational stimulus, the faster properties are inherently less economical in opposing gravity, and, thus, the muscle fibers become more fatigable when contracting against a load for long durations (Caiozzo et al. 1996).

e) Metabolic Processes

In contrast to the contractile apparatus, studies on various rodent skeletal muscle metabolic enzymes have revealed a variety of responses with no clear-cut adaptive changes in oxidative enzyme expression (Baldwin 1996a; Chi et al. 1992; Musacchia et al. 1992; Ohira et al. 1992; Roy et al. 1996a). These observations are consistent with the results of studies focusing on mitochondrial function after 9 days of spaceflight in which no reduction in the capacity of skeletal muscle mitochondria to metabolize pyruvate (a carbohydrate derivative) (Baldwin et al. 1993) was observed. These analyses were performed under state 3 metabolic conditions, i.e., non-limiting amounts of substrate and cofactors to simulate an energy turnover demand similar to that of high-intensity exercise (Baldwin et al. 1993). However, when a fatty acid substrate was tested, a reduction in the capacity of different muscle types to oxidize the long-chain fatty acid palmitate was observed (Baldwin 1996a; Baldwin et al. 1993). This latter finding is in agreement with the observation that muscles exposed to spaceflight increase the level of stored lipid within their myofibers (Musacchia et al. 1992). Additionally, use of the metabolic pathway for glucose uptake is increased in muscles undergoing HS (Musacchia et al. 1992). Thus, while the enzyme data are equivocal, it appears that in response to states of unloading, some shift in substrate preference may occur whereby carbohydrates are preferentially utilized based on utilization capability. If this is indeed the case, there could be a greater tendency for muscle fatigue if the carbohydrate stores become limited during prolonged bouts of EVA activity.

f) Functional Correlates of the Alterations in Muscle Mass and Contractile Phenotype in Response to Spaceflight

Stevens and associates (Stevens et al. 1993) reported that in isolated single-fiber analyses, deficits in force generation capacity were found along with a reduced sensitivity to calcium stimulation. Similar observations occurred for both slow and fast ankle extensor fibers after 14

days of spaceflight. This study focused on the force-generating aspects of muscle fibers. It appears that only two additional studies have been conducted to examine the effects of spaceflight on rodent skeletal muscle functional properties using a more comprehensive set of analyses. One project was carried out for 6 days (Caiozzo et al. 1994), while the other involved a 2-week flight (SLS-2) (Caiozzo et al. 1996). In both studies, the measurements focused on the force-velocity properties, which define the limits of functional capacity of the muscle. These studies were conducted on the soleus skeletal muscle, in which slow-twitch myofibers predominate, because of the dynamic changes in fiber morphology and phenotype that were observed in the studies summarized above. Analyses on the animals were initiated within 6 hours of return from spaceflight. The findings showed that the maximal strength of the muscle, as studied in situ using a computer-programmed ergometer system, was reduced by 24% after the 6-day flight and 37% after the 14-day flight (Caiozzo et al. 1996). These changes were consistent with the degree of atrophy observed at both the gross and single-myofiber levels. Additionally, shifts occurred in the force-frequency response of the soleus in the flight animals, suggesting a switch to a faster contractile phenotype. Maximal shortening velocities were increased by 14% and 24% in the 6- and 14-day spaceflight groups, respectively. These intrinsic increases in shortening speed were attributed, in part, to the de novo expression of the fast type IIx MHC in many of the slow muscle fibers. In contrast, both work- and power-generating capacities of the flight-induced atrophied muscles were significantly decreased. Additionally, the resistance to fatigue was significantly decreased as well as the ability to sustain work and power output in response to a paradigm involving repetitive contraction output (Caiozzo et al. 1996; McDonald et al. 1992a). Similar findings have been observed using comparable analytical approaches involving the HS model (Diffee 1993; Fitts et al. 1986; Gardetto et al. 1989). Taken together, the findings clearly indicate that when skeletal muscles, especially those with a large proportion of slow myofibers, undergo both atrophy and remodeling of the contractile phenotype, the functional capacity of the muscle is reduced along with its ability to sustain work output. If a sufficient mass of muscle tissue across several key muscle groups were similarly affected, the fitness of the individual would most likely be impaired when he/she is challenged with moderate-intensity exercise scenarios.

g) Are Atrophied Muscles Vulnerable to Injury?

Riley and associates (Riley et al. 1996; Riley et al. 1995) have provided an excellent synopsis of the structural integrity of mammalian muscle during the early stages after return from spaceflight. Their findings suggest that in atrophied slow types of skeletal muscle, there is no evidence of fiber damage when the muscles are taken from animals euthanized and processed during spaceflight. However, observations suggest that during the first 5-6 hours after spaceflight (the earliest time point at which the animals can be assessed), edema occurs in the target anti-gravity muscles, such as the soleus and adductor longus (AL) (Krippendorf and Riley 1993; Riley et al. 1996). This is thought to occur by increased blood flow to the muscles when they become initially reloaded in opposition to gravity. In addition, in certain regions of the AL, there is some indication of fiber damage based on histological analyses of the myofibril integrity and protein alignment in the sarcomere. While these observations were noted in ~2.5% of the fibers of the AL, they were not present in the soleus. Riley has proposed that the reason for the differential response between the two muscle groups is that weakened animals have altered their

posture and gait so that eccentric stress is placed on the AL, resulting in some fiber damage. Edema and fiber damage were not noted in another cohort of animals studied 9 days after landing (Krippendorf and Riley 1993; Riley 1998). However, in additional studies performed on both spaceflight and HS rodents (Krippendorf and Riley 1993; Riley 1998) in which 12 to 48 hours were allowed to pass before the muscles were analyzed, observations indicated that the normal cage activity induced significant lesions in the muscles after sufficient reambulation was allowed. These included eccentric-like lesioned sarcomeres, myofibrillar disruptions, edema, and evidence of macrophage activation and monocyte infiltration (known markers of injury-repair processes in the muscle) within target myofibers (Roy et al. 1991). These findings suggest that there is indeed a propensity for muscle injury secondary to the atrophy process that weakens the muscle and that, given the instability of the animal after spaceflight as described above, there is most likely a potential for injury if stressful stimuli are imposed on the muscle system before it can regain its proper structural and functional capability.

h) Cellular and Molecular Mechanisms of Muscle Atrophy in Response to Unloading Stimuli

As presented above, skeletal muscle atrophy involves an imbalance between the processes that control protein synthesis (also known as protein translation) and those that control protein breakdown. When the two processes are in synchrony, muscle mass is stable. However, if there is an imbalance such that the protein synthetic pathway activity is decreased relative to the degradation rate, muscle atrophy will occur. In the case of skeletal muscle atrophy in response to spaceflight or HS, a decrease in the capacity for synthesis and an increase in the processes that regulate degradation seem to occur, creating a rapid net degradation response to the unloading stimulus. On the basis of the available information, such a scenario is thought to involve the following chain of events. At the onset of unloading involving a wide range of models including spaceflight, a decrease in transcriptional and/or pre-translational activity occurs in skeletal muscle that affects the type I and IIa MHC genes as well as the actin gene (Baldwin et al. 2013; Caiozzo et al. 1996; Giger et al. 2009; Giger et al. 2005; Thomason et al. 1992). This results in a reduced level of both pre-mRNA and mRNA pools (the latter being a substrate for protein translation) for these three proteins. Together, MHC and actin provide the bulk of the myofibril fraction that accounts for most of the protein in the muscle cell. Concomitantly, a decrease occurs in the activity of key protein kinase enzyme systems (constituting the PI3 kinase/akt/mTOR pathway), which regulates the protein synthesis apparatus controlling protein translation (Adams et al. 2007; Baldwin et al. 2013; Haddad et al. 2006a). This alteration, in combination with a smaller amount of mRNA substrate, collectively contributes to a reduction in the net capacity for protein synthesis. Occurring simultaneously with this process is the up-regulation of a set of genes that encode proteins that play a regulatory role in augmenting protein degradation. These genes include the myostatin gene (Adams et al. 2007; Haddad et al. 2006a), the atrogin 1 gene (Adams et al. 2007; Haddad et al. 2006a), and a gene called muscle ring finger protein, referred to as MURF (Haddad et al. 2006a). Myostatin is an antigrowth transcription factor that is thought to negatively modulate the genes that promote growth. Atrogin and MURF are E3 ligases that are responsible for ubiquinating target proteins to mark them for degradation in a system designated as the proteasome. Interestingly, this MURF protein has been reported to be a key regulator that specifically targets breakdown of the type I and type IIa MHC proteins

(Fielitz et al. 2007). As a result of the reduction in net capacity for protein synthesis and the augmentation of protein degradation, a net loss of muscle protein in the muscle fiber occurs along with a change in the relative proportion of the MHC protein content, as available findings show that the faster MHC genes are up-regulated during muscle atrophy (Baldwin 1996b; Giger et al. 2009; Roy et al. 1996a). Thus, this results in a smaller, faster muscle phenotype, which is apparently more suitable for muscle performance in states of unloading. The chain of events described above must be blunted or reversed if the muscle is to perform optimally when faced with an increased gravitational stimulus upon returning to Earth or transitioning from low gravity (microgravity) to higher gravitational environments such as landing on the moon or Mars. It is apparent that the best strategy with which to accomplish this task is the use of a vigorous countermeasure program that provides a high level of mechanical stress to prevent the imbalance in protein expression that occurs when the muscle is insufficiently loaded for significant periods without an intervening anabolic stimulus.

i) Effects of Spaceflight on Non-Human Primates

To our knowledge, the only other species besides the rat that has been involved in spaceflight studies on skeletal muscle is the rhesus monkey. Two monkeys were flown in space for 14 days on the Bion 11 satellite. They were compared with ground-based vivarium control animals as well as a chair-restricted group that involved immobilization of the upper arm and shoulder. The results from these studies provided the following insights. Individual fibers (slow and fast) of the monkey displayed functional properties that were more closely aligned with those of human fibers than with those of rodents with respect to the observation that the fibers were larger but less powerful per unit of CSA than rodent fibers (Fitts et al. 1998; Fitts et al. 2000a). However, in pre- versus post-flight analyses of single fibers, slow fibers in both the slow-twitch soleus and triceps muscles underwent greater atrophy and reductions in force and power production than fast-twitch fibers. Additionally, transformations in the MHC profile indicated that there was a greater level of hybrid slow/fast fibers in the two different muscle groups (Fitts et al. 1998; Fitts et al. 2000a). Immobilization of the triceps muscle group produced similar responses, but the magnitude of change was much less than that in the spaceflight animals (Kelleher et al. 2014). Additional experiments were performed on these same animals to investigate locomotor activity before and after spaceflight via muscle electromagnetic and tendon force recordings, respectively. These experiments demonstrated that postural and locomotor control was compromised by spaceflight, as has been observed in humans (Fitts et al. 2000a; Fitts et al. 2000b; Fitts et al. 2010b; Recktenwald et al. 1999). These alterations were chiefly manifested as modified load-related cues, as reflected by the altered relative recruitment bias of flexor muscles versus extensors and fast versus slow motor unit pools. In an additional flight study (Cosmos Flight 2229) involving two rhesus monkeys, EMG recordings were obtained before, during, and after spaceflight (Roy et al. 1996b). These experiments were unique in that recordings obtained during spaceflight revealed a preferential shift in recruitment patterns favoring the fast medial gastrocnemius versus the synergistic slow soleus muscle, i.e., the normal recruitment pattern was reversed. This alteration was maintained well into the recovery stage after spaceflight, further suggesting a reorganization of the neuromotor system during and immediately after exposure to microgravity. Thus, it is apparent that skeletal muscle fibers of humans, monkeys, and rodents share similar patterns of myofiber alterations that, in the case of monkeys and humans, are also

linked to altered motor performance in response to different states of unloading, reduced usage, and return to an Earth gravitational environment.

2. Theme II: New Mechanistic Studies of Relevance to the Human Research Program

a) Effects of Spaceflight on Murine Skeletal Muscle Homeostasis

With the retirement of the space shuttle program, which enabled numerous studies concerning the role of gravity on skeletal muscle function and health in animal models, it is fortunate that Allen et al. (Allen et al. 2009) published an interesting article in 2009 on skeletal muscle gene expression in female mice (C57BL/6J) flown on the mid-deck in animal enclosure modules lasting 11 days and 19 hrs on the space shuttle Endeavor (STS-108/UF-1). It has been previously shown that spaceflight results in several adaptations to skeletal muscle, including both muscle atrophy and shifts toward faster muscle fiber types (see theme section I). To identify changes in gene expression concerning these types of alterations, the authors used both microarray expression analysis and real-time polymerase chain reactions to quantify shifts in mRNA levels in the gastrocnemius muscle from the flight mice versus normal gravity controls. Spaceflight data were also compared with the ground-based unloading model of hindlimb suspension, along with another group of pure suspension and one of suspension followed by 3.5 hours of re-loading to mimic the time between landing and euthanization of the spaceflight mice. Analysis of the microarray data revealed that 272 mRNAs were significantly altered by spaceflight, the majority of which displayed similar responses to those observed in response to HS, whereas reloading tended to counteract these responses. Several mRNAs altered by spaceflight were associated with muscle growth, including the phosphatidylinositol 3-kinase regulatory subunit p85 alpha, insulin response substrate-1, the fork head box O1 transcription factor, and MAFbx/atrogin1. Moreover, myostatin mRNA tended to increase, whereas mRNA of the myostatin inhibitor FSTL3 tended to decrease, in response to spaceflight. In addition, mRNA levels of the slow oxidative fiber-associated transcriptional co-activator peroxisome proliferator-associated receptor (PPAR)-gamma coactivator-1α and the transcription factor PPAR-α were significantly decreased in the spaceflight gastrocnemius muscle (which is indicative of a decrease in slow fiber gene expression). Therefore, these interesting results became a catalyst for numerous ground-based research themes using the HS model as delineated below.

b) The Rapid Kinetics of Muscle Wasting in Response to Ground-Based Unloading Models

In previous reviews, as summarized in the theme I section of this report, it was clearly demonstrated that weight-bearing muscle groups, such as the ankle extensors in rodents, are very sensitive to changes in loading state, especially during unloading conditions such as spaceflight and/or the model of HS. However, little is known of this process during the very early stages (hours) of unloading. Therefore, Giger et al. (Giger et al. 2009) characterized the dynamic changes in the unloaded rodent soleus muscle in vivo following a short bout of HS and tested the hypothesis that transcriptional events are rapidly impacted by the atrophic stimulus. In fact, their observations demonstrated that after only one day of HS, primary transcript (e.g., pre-mRNA and

mRNA) levels of skeletal alpha-actin and slow type I MHC genes were significantly reduced by more than 50% compared with ground control levels. The degree of decline for the mRNA expression of actin and type I MHC lagged behind that of the pre-mRNA after 1 day of HS, but by 2 and 7 days of HS, large decreases in mRNA for the two genes were observed. Although the faster MHC isoforms, IIx and IIb, began to be expressed in the soleus after 1 day of HS, a relatively significant shift in mRNA expression from the slow MHC isoform to the fast isoforms did not emerge until 7 days of HS.

Interestingly, one day of HS was sufficient to induce significant decreases in mRNA levels of putative signaling factors such as serum response factor (SRF), suppressor of cytokine signaling-3 (SOCS-3), and striated muscle activator of Rho signaling (STARS), although the transcription factors yin-yang-1 (YY1) and transcriptional enhancing factor-1 (TEF-1) were not as significantly affected. Interestingly, the protein levels of actin and type I MHC were significantly decreased after 2 days of HS, implying that myofibril degradation is also being impacted early on during the atrophic stimulus. These alterations suggest the following: 1) the synthesis side of the protein balance equation is rapidly down-regulated, whereas the degradation process is most likely enhanced (see below), during the early stages of unloading. If these alterations that are occurring in these animals models are also occurring in humans (e.g., astronauts), it becomes apparent that it is critical that countermeasures, such as resistance training, must be initiated in the early phase of exposure to microgravity environments.

c) Mechanisms of Slow to Fast MHC Gene Switching during Unloading: Role of Non-coding Antisense RNA

In previous sections of this report, we described that in response to unloading stimuli (HS model), there was a change in MHC gene expression whereby the slow-type I and faster type IIa genes were repressed while the fast type IIx and IIb genes were expressed *de novo* in the unloaded soleus muscle of rodents (Baldwin 1996b; Caiozzo et al. 1994; Caiozzo et al. 1996; Thomason and Booth 1990). Recall that the MHC gene family in striated muscle comprises at least eight members: two cardiac genes, alpha and beta, three adult fast MHCs (IIa, IIx, and IIb), two developmental MHCs (Embryonic and Neonatal), and one specialized type, i.e., the extraocular MHC (EO). Note that the slow cardiac beta MHC is the same as the type I MHC gene that is expressed in slow skeletal muscle fibers. As discussed in more detail elsewhere (Baldwin et al. 2013), these MHC genes are arranged into two clusters: 1) the cardiac MHCs on chromosome 15 in the rat, and 2) the skeletal muscle MHC cluster on chromosome 10. This gene clustering orientation and tandem organization has been conserved through millions of years of mammalian evolution. This conserved configuration raises questions as to whether this particular MHC gene alignment is of functional significance with respect to their patterns of regulation under different physiological states.

Recent evidence has implicated a non-coding RNA in the coordinated regulation of two positioned genes in tandem, which implicates the importance of genomic organization of these MHC genes in their coordinated regulation. For example, in 2003, Haddad et al. (Haddad et al. 2003) reported the novel discovery that in normal healthy rodent cardiac muscle, a naturally

occurring antisense RNA transcript to the cardiac Beta (type 1) MHC gene is involved in cardiac gene regulation, such that the alpha MHC isoform is normally primarily expressed under normal physiological conditions (Haddad et al. 2003). Interestingly, cardiac alpha and beta MHC isoforms are the products of two distinct genes that are organized in tandem in a head to tail position on the same chromosome in the order of beta → alpha (e.g., the beta gene is upstream of the alpha gene), and they are separated by a ~4.5-kb intergenic DNA space (Mahdavi et al. 1984). In the normal state, a long non-coding antisense RNA is transcribed from the DNA that is opposite to the MHC genes, creating a "beta antisense RNA". This antisense-beta sequence was implicated in MHC isoform gene regulation/switching (alpha MHC repression and beta expression enhanced) in the heart in response to both diabetes and hypothyroidism (Haddad et al. 2010; Haddad et al. 2008; Haddad et al. 2006b). Under conditions of both hypothyroidism and type I diabetes, the antisense Beta fragment was repressed, allowing the beta MHC gene to dominate cardiac beta MHC compared with the normal heart. Given these findings, studies were subsequently performed on skeletal muscle to ascertain whether the non-coding antisense RNA expression in slow and fast skeletal muscle contributes to the patterns of MHC gene expression in response to unloading stimuli.

In 2006, Pandorf et al. (Pandorf et al. 2006) published a paper on a study that investigated type II MHC gene regulation in slow type I soleus muscle fibers undergoing a slow to fast MHC transformation in response to seven days of spinal isolation (SI), a model of inactivity that induces atrophy similar to HS (Baldwin et al. 2013). Transcriptional products of both the sense and antisense strands were examined across the IIa-IIx-IIb MHC gene locus as depicted in the paper by Baldwin et al. (Baldwin et al. 2013). Results showed that the mRNA and pre-mRNA of each MHC gene had a similar response to the SI stimulus, suggesting the regulation of these three genes at the transcriptional level. In addition, detection of a previously unknown antisense strand transcription occurred that produced *natural antisense transcripts* (NATs). RT-PCR mapping of the RNA products revealed that the antisense activity resulted in the formation of three major products: aII, xII, and bII NATs, i.e., antisense products of the IIa, IIx, and IIb genes, respectively. Thus, the key observation of this experiment was that the SI-induced inactivity caused a marked inhibition of both the slow type I and type IIa genes along with upregulation of both the IIx and IIb genes. Therefore, the inactivity model of SI negatively impacts transcription of the type I MHC gene by inhibiting its promoter (Giger et al. 2009; Pandorf et al. 2006) and induces antisense aII NATS that primarily repress transcription of the IIa MHC gene, thereby creating a switch from slow type I/IIa predominance to a fast IIx fiber of the normally slow soleus muscle. Importantly, this observation explains the existence of type I/IIx hybrid fibers reported previously by Caiozzo et al. (Caiozzo et al. 1996), as presented in the earlier section of this review.

d) Mechanisms of Slow to Fast MHC Gene Switching During Unloading: Role of Epigenetic Modification of MHC Gene Histones

Recent advances in chromatin biology have enhanced our understanding of gene regulation, especially that of the motor protein MHCs. It is now widely appreciated that gene regulation is dependent on post-translational modifications to the histones that package genes in the nucleus of the cell. Active genes are known to be associated with acetylation of histones (H3ac) and

trimethylation of lysine 4 in histone H3 (H3K4me3). Using chromatin immuno-precipitation (ChIP), Pandorf et al. (Pandorf et al. 2009) examined histone modifications at the MHC genes expressed in fast versus slow fiber-type skeletal muscle, as well as in a model of muscle unloading (HS), which results in a shift to fast MHC gene expression in slow muscles. Both H3ac and H3Kme3 varied directly with the transcriptional activity of the MHC genes in fast fiber type plantaris and slow fiber-type soleus. During MHC with muscle unloading, histone H3 at the type I MHC becomes de-acetylated in conjunction with the down-regulation of that gene, while upregulation of the fast type IIx and IIb MHCs occurs in conjunction with enhanced H3ac in those MHCs. Enrichment of H3K4me3 is also increased at the type IIx and IIb MHCs when these genes are induced by muscle unloading. Downregulation of IIa MHC, however, was not associated with corresponding loss of H3ac or H3K4me3. These observations demonstrate the feasibility of using the ChIP assay to understand the native chromatin environment in adult skeletal muscle and also suggest that the transcriptional state of the types I, Ix, and IIb genes are sensitive to histone modifications both in different muscle fiber-types and in response to altered loading states.

e) Strategies for Ameliorating the Rapid Kinetics of Muscle Wasting

In 2009, Susan Kandarian's research group (Van Gammeren et al. 2009) using the HS model of unloading demonstrated that nuclear factor kappa B (NF-kB) signaling is necessary for the enhanced degradation that occurs during the early stage of unloading-induced atrophy. Importantly, when this factor was inactivated, the atrophy process was inhibited, suggesting that NF-kB plays a major role in the degradation cascade of the myofibril network during unloading.

In a follow-up study (Jackman et al. 2012), the Kandarian group, using ChiP-gene sequencing technology, revealaed that Bcl-3, an NF-kB transcriptional activator, is required for atrophy, and this factor also binds to the promotors of several genes collectively involved in muscle wasting. By means of bioinformatics analysis of ChiP-sequencing data, they discovered that Bcl-3 directs transcription networks that include many E3 ligases associated with the proteasomal protein degradation network, including that of the N-end rule pathway. These findings are important because they could facilitate the development of a process to either slow down or prevent the acceleration of muscle wasting by inhibiting this critical pathway.

For example, atrogin-1 and MurF1 are muscle-specific ubiquitin ligases that play a pivotal in protein degradation by targeting myofibril protein for degradation during states of unloading. Interestingly, Maki et al. (Maki et al. 2012) tested the hypothesis that branched-chain amino acids (BCAAs) inhibit atrogein-1 and MuRF1 and have a protective effect on disuse muscle atrophy. To test this hypothesis, they used the HS Model. Their findings showed the following: 1) HS significantly reduced soleus muscle weight and the CSA of soleus muscle fibers; 2) branched chain amino acid administration significantly reversed the HS-induced decreased in fiber CSA; and 3) while HS increased expression of atrogin1 and MuRF1, which are involved in muscle atrophy, branched-chain amino acid attenuated the increase in atrogen-1 and MuRF1 in the soleus muscles. Thus, further studies on this important finding are warranted.

In another interesting study, Derbre et al. (Derbre et al. 2012) developed a strategy to determine the mechanism by which xanthine oxidase (XO) causes unloading-induced muscle atrophy in rats via HS along with its potential prevention by allopurinol, a well-known inhibitor of XO, and a key therapeutic factor in preventing gout. For this purpose, the authors studied one of the main redox-sensitive signaling cascades involved in unloading-induced atrophy, i.e., p38 MAP kinase, along with the expression of two primary muscle-specific E3 ubiquitin ligases involved in proteolysis, e.g., atrogin-1 and MuRF-1. Their findings clearly showed that HS induced a significant increase in XO activity protein expression of the antioxidant enzymes CuZn, SOD, and catalase in skeletal muscle. The most significant finding in this paper involved the inhibition of XO with allopurinol and significantly reduced soleus muscle atrophy, along with inhibition of atrogin-1 and MuRF-1 expression, which are pivotal factors of myofibril degradation in the proteasome. As stated above, more research on this potential countermeasure is warranted.

f) Exercise Strategies to Counteract Muscle Atrophy during Early and Long-Term Stages of Unloading

In the last eight years, to the authors' knowledge, few studies were carried out to ascertain the mechanisms for counteracting the rapid atrophy of animal skeletal muscle as presented above. In 2006, Haddad et al. (Haddad et al. 2006a) performed a study to test the hypothesis that an isometric resistance training paradigm targeting the medial gastrocnemius muscle of adult rodents is effective in preventing muscle atrophy during the early stages of unloading by maintaining normal activation of the insulin receptor substrate-1 (IRS-1)/phospho-inositide-3 kinase (PI3K)/Akt signaling pathway. This pathway has been shown to simultaneously create an anabolic response while inhibiting processes that up-regulate catabolic processes involving expression of key enzymes in the ubiquitination of protein for degradation of the myofibril network. The findings of this study revealed the following: during the 5 days of unloading, 1) an absolute medial gastrocnemius muscle weight reduction of 20% occurred, but muscle weight corrected to body weight was not different from that of normal weight-bearing controls; 2) myofibril concentration and content were decreased; and 3) a robust isometric training program, known to induce a hypertrophy response, failed to maintain the myofibril protein content. This response occurred despite fully blunting the increases in the mRNA for atrogen-1, MURF-1, and myostatin, e.g., sensitive gene markers that activated the catabolic state. Analyses of the IRS-1/PI3K/Akt markers indicated that the abundance of IRS-1 and phosphorylation state of Akt and p70S6 kinase were decreased relative to that of normal control rats, and the resistance training failed to maintain these signaling markers at normal regulatory level. These findings were insightful and suggest that to fully prevent muscle atrophy responses affecting the myofibril system (which is the primary target of atrophic stimuli) during unloading, the volume of mechanical stress must be augmented sufficiently to maintain optimal activity of the IRS-1/PI3K/Akt pathway to provide an effective anabolic stimulus for the target muscle.

Based on the above information, Adams et al. (Adams et al. 2007) undertook a study to determine whether resistance training, with an increased volume (3-second contractions) along with the incorporation of both static and dynamic contractile components, would be effective in preventing rapid unloading-induced atrophy. Rats were exposed to 5 days of muscle unloading

via HS. During that time, one leg received electrical stimulated resistance exercise (RE) that included isometric, concentric, and eccentric contraction phases. The results of this study indicate that this combined-mode RE provided an anabolic stimulus sufficient to maintain the mass and myofibril content of the trained but not the contralateral medial gastrocnemius (MG) muscle. Relative to the contralateral MG, the RE stimulus increased the amount of total RNA (indicative of translational capacity) as well as mRNA for several anabolic/myogenic markers, such as insulin-like growth facor-1, myogenin, myoferlin, and collogen III-alpha-1, and decreased that of myostatin, a negative regulator of muscle fiber size. The combined-mode RE also increased the activity of anabolic signaling intermediates such as p70S6 kinase (constituents of the IRS-1/PI3K/Akt pathway). These results indicate that a combination of static- and dynamic-mode RE of sufficient volume provides an effective stimulus to stimulate anabolic/myogenic mechanisms to counter the initial stages of unloading-induced muscle atrophy.

In the context of the above findings, Dupont et al. (Dupont et al. 2011) also studied the role of the IRS-1/PI3K/Akt pathway during hindlimb unloading of the soleus and fast extensor digitorim longus muscles over a span of 7, 14, and 28 days in the context of performing chronic low-frequency stimulation (soleus) to maintain the contractile phenotype and muscle mass. The unloaded muscle induced a down-regulation of the Akt pathway and an up-regulation of the catabolic FOXO1 and muscle-specific MURF-1, i.e., markers indicative of a catabolic state. Chronic low-level stimulation of the soleus muscle failed to maintain muscle mass at all the time points examined but did maintain the slow MHC phenotype in the soleus (e.g., non-switching of slow to fast MHC phenotype). These findings indicate the importance of loading the target muscles to maintain a bias of anabolic stimuli relative to the catabolic state that prevailed with the low-frequency stimulation. One of the primary findings of the low-frequency stimulation model is that it actually induces muscle atrophy when combined with normal loading conditions. Thus, this type of countermeasure is counterproductive to maintaining muscle mass.

g) Does Aerobic Exercise Serve As a Protective Preconditioning Stimulus for Unloading-Induced Atrophy?

Recently, Fujino et al. (Fujino et al. 2009) conducted a study to determine if 25 minutes of aerobic treadmill running provides a protective precondition stimulus to counteract the deleterious effects of hindlimb unloading of adult male rats. The following groups were studied: a ground-based control group, a 2-week HS group, and a group that performed 25 minutes of aerobic exercise prior to undergoing two weeks of HS. The results of this study were quite surprising. As expected, soleus mass, maximum tetanic tension, myofibrillar protein content, muscle fatigue resistance, and percent of type I MHC were decreased in unloaded rats compared with the ground-based control. In addition, markers for the cathepsin, calpain, caspase, and ATP-ubiquitin-proteasome proteolytic pathways were increased in the suspension group compared with the ground controls. However, the preconditioning endurance exercise bout attenuated all of the detrimental changes associated with HS and also increased the expression of heat shock protein 72. The authors concluded that their findings indicate that exercise pre-conditioning may be an effective countermeasure to buffer the detrimental effects of chronic decreases in activation and loading levels on skeletal muscle, and HSP 72 may be one mechanism associated

with these responses. If these findings can be verified by additional studies, this countermeasure strategy could open up a new avenue in terms of preventing the various deleterious alterations that impact animal and human skeletal muscle in unloading environments.

h) Role of the "Myonuclear Domain" in the Regulation of Muscle Cell Size

One of the unique features of the skeletal muscle cell is that it is the only cell type that expresses multiple nuclei in each fiber cell. It has been thought that each nucleus in any given fiber manages a specific volume of cytoplasm (Bruusgaard et al. 2012). Over the years, several studies have provided evidence that the nuclear domain is not static. Rather, during unloading conditions, myonuclei are reduced as the size of the fiber is reduced (Allen et al. 1997)**.** Additionally, the opposite occurs when the myofiber becomes hypertrophied in response to anabolic stimuli (Allen et al. 1997). Recently, Bruusgaard et al. (Bruusgaard et al. 2012) challenged this long-standing hypothesis when they demonstrated that atrophy induced by HS involving adult female rats does not lead to loss of nuclei despite a strong increase in apoptotic activity of other types of nuclei within the muscle tissue (e.g., non-muscle type nuclei). Thus, in the authors' view, HS is similar to other atrophy models, such as denervation, nerve impulse block, and antagonist ablation. The authors discuss several flaws concerning the different studies published to date that can be attributed to difficulties in separating myonuclei from other non-muscle nuclei surrounding the myofibers along with systematic differences in passive properties between normal and unloaded muscle. During reloading after HS, a normal re-growth was observed, which has been believed to be accompanied by recruitment of new myonuclei from satellite cells expressed outside of the fibers. However, in this study (Bruusgaard et al. 2012), the authors observed that reloading led to a 59% increase in CSA and that the fiber size was completely restored to normal pre-HS sizes, with no parallel increase in the number of myonuclei incorporated into the fibers. Thus, radial regrowth seems to differ from *de novo* hypertrophy in that nuclei are only added from *de novo* hypertrophy when muscle fibers are induced to undergo an increase to a new and larger muscle fiber size. Clearly, these findings are important and deserve further scrutiny and follow-up studies given the importance of understanding the cellular mechanisms of both atrophy and hypertrophy processes.

i) Why are Slow-Type Muscles More Sensitive to Unloading-Induced Atrophy than Fast-Type Muscles?

It is apparent that slow-type muscles, such as the soleus, vastus intermedius, and adductor longus, are more sensitive to unloading conditions such as space flight/HS than their fast-type counterparts, such as the plantaris and medial gastrocnemius (Baldwin 1996b). The mechanisms impacting this differential response are essentially unknown. However, a recent study published in 2012 by Bortoloso et al. (Bortoloso et al. 2013) provided some important information that may shed light on this interesting phenomenon. They studied the expression of a new and diversified family of proteins called "Homers". These homer isoforms (e.g., 1b/c and 2a/b) were characterized in fast- and slow-twitch skeletal muscle in rats and mice. Homer 1b/c was identical irrespective of the muscle type, whereas Homer 2a/b was characteristic of the slow-twitch

phenotype such as the soleus. Transition between Homer isoforms was studied in two established experimental models of atrophy, i.e., after denervation and hindlimb unloading in slow-twitch skeletal muscle of the rat. No change in Homer 1b/c was observed in up to 14 days of denervation, whereas Homer 2a/b was found to be significantly decreased by 70 and 90% at 7 and 14 days of denervation, respectively, which paralleled the reduction in muscle mass. Seven-day HS decreased Homer 2a/b by 70%. Interestingly, reconstitution of Homer 2 by *in vivo* transfection of denervated soleus muscle allowed partial rescue of the atrophic phenotype with respect to muscle mass, muscle fiber size, and ubiquitination. The counteraction effects of exogenous Homer 2 were mediated by down-regulation of MURF-1, Atrogen-1, and Myogenin, i.e., all genes known to be up-regulated at the onset of atrophy. Thus, the present data show that 1) down-regulation of Homer 2 is an early event of slow muscle atrophy, and 2) Homer 2 participates in the control of ubiquitination and ensuing proteolysis via transcriptional down-regulation of MuRF1, Atrogen 1, and Myogenin. Therefore, Homers are key players of skeletal muscle plasticity, and Homer 2 is required for trophic homeostasis of slow-twitch muscle.

j) Newly Discovered Genes that Regulate Muscle Mass Stability and Atrophy Mechanisms

In the last five years, several genes have been discovered that play a major role in determining the stability of muscle mass homeostasis. For example, the Scott Kimball/Leonard Jefferson group (Kelleher et al. 2014; Kelleher et al. 2013) discovered the role of REDD1 and REDD2 genes as pivotal regulators of the MTORC1 anabolic signaling pathway in the models of limb immobilization, limb suspension, and bed rest that impacts muscle atrophy. Studies were performed on male rats that were subjected to unilateral hindlimb immobilization for 1, 2, 3, or 7 days or served as non-immobilized controls. Following overnight fasts, rats received either saline or L-leucine by oral administration as a nutrient stimulus. Hindlimb skeletal muscles were processed and analyzed for the rate of protein synthesis, MRNA expression, phosphorylation state of key proteins in the mTORC1 signaling pathway, and the mTORC1 signaling repressor REDD1/2. In the basal state, mTORC1 signaling and protein synthesis were repressed within 24 hrs in the soleus muscle of the immobilized compared with the non-immobilized hindlimb. These responses were accompanied by a concomitant induction of the expression of REDD1/2. In contrast with the L-leucine stimulus, there was elevation of similar magnitude in mTORC1 stimulus in both the immobilized and non-immobilized muscle, which was accompanied by the phosphorylation of the 70-kDa ribosomal protein S6 kinase in only the nutrient stimulus group. These findings suggest that signaling through mTORC1 becomes impaired in response to immobilization by induction of REDD1/2, causing a defective p70S6 kinase enzyme.

In a follow-up study (Kelleher et al. 2014), the authors studied the mechanism of why immobilized skeletal muscle fixed in a shorten position displays disuse atrophy whereas it does not exhibit atrophy when fixed in a stretched position. They tested the hypothesis that skeletal muscle in the stretched position would be protected from gene expression changes known to be associated with disuse atrophy, such as REDD1/2. To test this hypothesis, male rats were subjected to unilateral hindlimb immobilization for 3 days with the soleus fixed in either a shortened or stretched position, with results compared with the contralateral non-immobilized muscle. Soleus immobilized in a shortened position exhibited disuse atrophy, attenuated rates of

protein synthesis, attenuated mTORC11 signaling, and induced expression of genes encoding REDD1, REDD2, Atrogin-1, and MuRF1 (markers of protein degradation). In contrast, immobilization in the stretched position prevented these changes, as it yielded no difference in muscle mass, rates of protein synthesis, mTORC1 signaling, or expression of genes encoding REDD1, REDD2, Atrogin-1, and MuRF1. Thus, muscle immobilized in the non-stretched position leads to induction of gene expression of REDD1, REDD2, and the atrogenes that induce protein degradation.

Complementing the findings presented above, Nakao et al. (Nakao et al. 2009) reported that skeletal muscle atrophy caused by unloading is characterized by both a decreased responsiveness to myogenic growth factors (e.g., insulin-like growth factor 1 (IGF-1)) and increased proteolysis. This occurs via the induction and activation of the ubiquitin ligase Cbl-b. Upon induction, Cbl-b interacts with and degrades the IGF-1 signaling intermediate IRS-1. In turn, the loss of IRS-1 activates the FOXO3-dependent induction of atrogen-1/MAFbx, a dominant mediator of proteolysis in atrophying muscle. Cbl-deficient mice were resistant to unloading-induced atrophy and muscle function loss. Furthermore, a pentapeptide mimetic of tyrosine (608)-phosphorylated IRA-1 inhibited Cbl-b mediated IRS-1 ubiquitination and strongly decreased the Cbl-b-mediated induction of atrogen-1/MAFbx. These observations indicate that the Cbl-b-dependent destruction of IRS-1 is a critical dual mediator of both the increased protein degradation and reduced protein synthesis observed in unloading-induced muscle atrophy. Additionally, the inhibition of Cbl-b-mediated ubiquitination may be a new therapeutic strategy for treating unloading-mediated muscle atrophy.

k) Novel Insights into Isolated C2C12 Myocytes in Models of Atrophy

A recent study by Kazi et al. (Kazi et al. 2011), using isolated C2C12 myocytes in cell culture, has provided very interesting results regarding the role that Deptor plays in protein metabolism. Deptor is an mTOR binding protein that is thought to inhibit mTOR-S6Kinase signaling during protein synthesis. The authors postulated that by knocking down Deptor expression in C2C12 myocytes, mTOR activity and protein synthesis would occur. Deptor knockdown was achieved by sing lentiviral particles containing short hairpin (sh) RNA targeting the mouse Deptor mRNA sequence. Knockdown reduced Deptor mRNA and protein content by 90%, which increased the phosphorylation of mTOR kinase substrates, 4E-binding protein-1 and S6Kinase1, and concomitantly increased protein synthesis along with cell size. Interestingly, Deptor knockdown (50% reduction) by electroporation in the gastrocnemius of C57/BL6 mice did not alter weight or protein synthesis in the control muscle. However, Deptor knockdown prevented atrophy by day 3 of hindlimb immobilization by increasing protein synthesis. These findings support the notion that Deptor is an important regulator of protein metabolism in myocytes and demonstrate that decreasing Deptor expression *in vivo* is sufficient to ameliorate muscle atrophy.

l) Is Loss of Skeletal Muscle Mass and Function Experienced by Astronauts and Animals during Space Flight Impacted by Ionizing Radiation?

It is unknown whether the loss of skeletal muscle and function experienced by astronauts during space flight could be augmented by ionizing radiation (IR), such as low-dose high-charge and energy (HZE) particles of low-dose high-energy proton radiation. Shtifman et al. (Shtifman et al. 2013) performed a study on adult mice that underwent whole-body irradiation with either a single dose of 15 cGy of 1 GeV/n Fe particles or 90 cGy protons at 1GeV/n. Both ionizing radiation types caused alterations in the skeletal muscle cytoplasm Calcium-2 (Ca2) homeostasis. Fe-particle irradiation also caused a reduction of depolarization-evoked Ca2 release from the sarcoplasmic reticulum. The increase in the calcium content was detected as early as 24 hr after Fe-particle irradiation, while the effects of proton irradiation were only evident at 72 hours. In both instances, calcium content returned to baseline levels at day 7 after irradiation. Neither un-irradiated controls nor proton-irradiated samples exhibited such a phenotype. Protein analysis revealed a significant increase in the phosphorylation of AKt, Erk1/2, and rpS6K on day 7 in Fe-particle-irradiated skeletal muscle, but not in proton- or un-irradiated skeletal muscle, suggesting the activation of pro-survival signaling. These findings suggest that a single low-dose Fe-particle or proton exposure is sufficient to affect Ca2 homeostasis in skeletal muscle. However, only Fe-particle irradiation led to the appearance of central nuclei and activation of pro-survival pathways, suggesting an ongoing muscle damage/recovery process. These findings suggest the need to investigate the effects of chronic radiation exposure on the steady-state health of the muscle system.

m) Future Directions

Based on the animal studies and unique ground-based models of unloading that certainly complement the spaceflight findings reported herein on skeletal muscle homeostasis, it is obvious that NASA needs to continue sponsoring relevant animal research programs. This is evident by the recommendations provided in the **National Research Council of the National Academies in their 2011 Report: Recapturing a Future for Space Exploration. In Chapter 6: Animal and Human Biology**, which included a series of recommendations to establish animal research on the International Space Station (ISS). Such a program appears to be in the planning/implementation stages and will hopefully have been implemented prior to the publication of the next Evidence Report: Risk of Impaired Performance Due to Reduced Muscle Mass, Strength, and Endurance.

3. Summary of Animal Experiments

The use of animal research models during space flight and in space flight analogs has been an invaluable tool in better understanding the effects of unloading-induced skeletal muscle adaptation. Animal research has corroborated several human space flight findings that have been observed in far more limited cohorts, thus giving support to the physiological models. For example, animal research has shown that space flight primarily affects postural muscles, larger fibers are generally more susceptible to muscle atrophy than smaller fibers, and slow fibers are

more affected than fast fibers. Animal research has also been at the cutting edge of our understanding of how space flight negatively affects muscle mass. Animal models have been used to identify the key molecular pathways that regulate muscle protein synthesis as well as protein degradation in mammalian skeletal muscle. Animal models have also shown where there are lesions in the normal regulation of these pathways in response to space flight or space flight analogs. This information may be vital in moving forward in developing effective new countermeasures that directly target the regulators of muscle mass that are most affected by space flight and understanding why other countermeasures may fall short. Animal research also plays an important role in the comprehensive study of the effects of space flight on skeletal muscle by providing a model for which human experiments cannot be directly tested (e.g., space radiation studies). In short, animal space flight research has provided both the corroborating and leading-edge scientific knowledge base needed to adequately mitigate the effects of reduced muscle mass, strength, and endurance in skeletal muscle.

V. Computer-based Simulation Information

Limited work is currently being pursued to utilize computational modeling to predict the influence of microgravity or the efficacy of countermeasures on skeletal muscle mass and function. NASA has a dedicated effort in this area under the Digital Astronaut Project (DAP) (White 2007). The goal of the DAP is to develop and implement well-validated computational models to predict and assess spaceflight health and performance risks, and enhance countermeasure development. To ensure the computational models appropriately represent the physiologic process that may play a role in spaceflight, the DAP works closely with NASA's subject matter experts in muscle and exercise physiology. Given the early stage of this work, peer-reviewed citations do not exist regarding the use of these models to predict the loss of skeletal muscle mass and function in a microgravity environment or to predict the efficacy of exercise countermeasures.

VI. Risk in Context of Exploration Mission Operational Scenarios

It must be stated at the outset that the risk(s) related to loss of skeletal muscle mass, strength, and endurance depends not only on the level of loss but also on the starting point and the relative physiological expense required to successfully complete a requisite set of tasks within a fixed period. Thus, a crewmember must be capable of completing a task before being exposed to microgravity, the amount of functional loss cannot be allowed to fall below the level needed to successfully complete all assigned tasks, and the physical performance requirements for completion of the tasks should be known. Without information relating to the physical performance requirements of tasks, it is not possible to determine the risk of failure. Additionally, if a task could not be completed by a crewmember before microgravity exposure, it can reasonably be stated that the risk of failure during a mission is 100%. However, even if the crewmember has the capability to complete every possible task, a composite of the tasks to be completed over a finite period presents an entirely different requirement because it may be possible to select a composite of tasks to be completed within a work period that exceeds the capabilities of a single crewmember or perhaps every crewmember. Additionally, all possible contingencies that may arise must be considered so that a crewmember will be able to deal with

such off-nominal scenarios even near the end of a duty day. Thus, even an approach as basic as thoughtful scheduling of daily tasks could serve to help mitigate risk.

From the above discussion, several important items emerge that must be known with respect to the risks related to loss of skeletal muscle mass, strength, and endurance. These include:

- Baseline level of crewmember functional performance (e.g., strength, endurance, level of functional performance)
- Magnitude of functional loss from baseline at any point during the mission
- Physiological demand of a task or set of tasks to be completed
- The time period in which the tasks should be performed
- All possible contingency events that could have an impact on functional performance
- Any other interfering conditions that could affect functional performance (e.g., nutritional and psychological status, EVA suit specifications, equipment malfunction or failure, illness, injury).

An indication of the importance of individual baseline performance is available based on an illustrative example from the EDOMP program. Losses in trunk flexor and extensor strength were greater for the crewmembers who exercised on the Shuttle treadmill during flight than for the crewmembers who did not exercise during their mission (see Figure 7). Although this seems counterintuitive, it is likely that crewmembers who chose to exercise during flight did so because they exercised regularly as part of their daily routine before flight. Because they were at a higher level of fitness than their non-exercising crewmates, the hypokinesia of spaceflight represented a greater relative reduction in loading for them and, thus, may have increased their vulnerability to losses during flight. However, what cannot be ascertained from percent change data are absolute strength levels. For instance, exercising crewmembers who lost twice as much abdominal and back muscle strength as their non-exercising counterparts could still have greater strength in those muscles if they started off twice as strong as their non-exercising colleagues.

With respect to future missions involving humans, lunar sortie missions will probably represent the lowest risk of the currently planned missions and will likely be no greater in risk than the Apollo missions (at least with respect to skeletal muscle performance) unless unusual surface operations are planned that differ markedly from Apollo lunar surface operations. The longest cumulative time of lunar surface EVA by a crew during the Apollo Program was approximately 22 hours (combined from 3 separate days), and the longest total duration of the crew on the lunar surface was approximately 75 hours during the sixth and final Apollo mission (Apollo 17).

The answer to the question of whether exercise equipment should be available to crewmembers for short missions to the Moon and back is actually an easy one, and the answer is a resounding "Yes." During some of the Apollo missions, a small, lightweight device called the "Exer-Genie," which required no external power, was made available to crewmembers (see Figure 1), and they were encouraged to use it. Specific comments from the Apollo crewmembers collected during the recent "Apollo Summit" are particularly relevant (Scheuring 2007) and can be summarized as follows:

- Exercise is not necessary on a "short" trip, and crews did not feel that they suffered "noticable" deconditioning; however, they did demand that exercise capability be

available as much as possible for "rest and relaxation" for ALL phases of the mission. The exercise device was used by all crewmembers with varying amounts and intensities. Lunar surface crews (maximum time spent on surface operations [EVAs] was 22 hours during the 75-hour stay of Apollo 17) felt that their activities on the lunar surface provided enough exercise for a short-duration mission but would have welcomed a simple, robust device for stretching and forearm exercise. (Note: The Exer-Genie remained in the Command Module with the Command Module Pilot; it did not accompany the two astronauts who descended to the lunar surface in the lunar excursion module.)

- Apollo crewmembers felt that crew surgeons and mission planners should not hard-schedule exercise prescriptions for such short-duration missions but rather allow the crew to perform them at their leisure.
- They stated that a more robust and lightweight piece of in-flight exercise equipment is needed than flown during Apollo. The Exer-Genie was limited, its ropes were friable, and the device generated a lot of heat and odor, so an alternative exercise device is needed.
- Most crewmembers felt that the pre-mission timeline should provide adequate time to maintain musculoskeletal strength and stamina. Some astronauts attributed their capabilities on the lunar suface to pre-mission training because in some cases, more force was needed on the lunar surface while wearing the EVA suit than was needed in 1 G on Earth.
- The crew felt that Exer-Genie or an alternative was definitely needed, and because of a fear that they would break it, they actually tapered off from intense use to save it for use in reconditioning on the return trip before re-entry.
- The strongest comment was that "as many exercise capabilities as possible should be built into all future vehicles" because they will get used, and the crew further felt that exercise capability throughout flight was critical and that a variety of exercises should be provided.

Lunar outpost missions will present a greater challenge than shorter "sortie" missions, but with respect to the current risk topic, they probably represent risks similar to those experienced on the ISS. Lunar gravity, although approximately 1/6 that of Earth gravity, is still more conducive to providing sufficient loading to maintain muscle mass and function than is microgravity. Exercise regimens and hardware will certainly be required, not only for countering muscle atrophy but also for the reasons stated by Apollo astronauts above as well. The amount of exercise that is needed and the proper way to implement it are certainly knowledge gaps that will require innovative research to be filled. Part of this research will unquestionably help to define the level of risks to which crews will be exposed but will also be helpful in properly mitigating those risks.

Without a doubt, transport between the Earth and Mars as well as the return trip represent the greatest risks to humans encountered in the history of human spaceflight. Notwithstanding the risks of radiation exposure, deterioration of the musculoskeletal system must be prevented or a mission to Mars (and back) will not be successful. Highly refined exercise protocols and robust exercise equipment and methods to monitor functional capacity are mandatory for

mitigation of the risks inherent in long-duration exposure of humans to microgravity. A significant challenge will be to provide the above within the current design of the crew exploration vehicle (CEV), which provides only a small amount of space for equipment and crew. The cramped confines will afford little room for stretching or exercise. Modest or no power for equipment and a human life support system whose design may be marginal to support a full complement of exercise by efficiently dealing with the heat, water vapor, and carbon dioxide that are byproducts of human exercise are additional challenges that must be overcome.

Knowledge gained during lunar outpost missions will be highly relevant to successful establishment of a Martian outpost. If the challenges posed by the long transit to Mars and the extended period of microgravity exposure can be met, the outpost phase should represent a much lower risk by comparison, as lunar outpost experience will have allowed significant opportunity to develop risk-mitigation strategies for this phase. The gravitational environments are similar; in fact, the Martian gravity field, which is greater than that of the Moon, will provide a less formidable setting. However, capability to provide sufficient exercise capacity during the Martian outpost phase is essential in preparing the crew for a long-duration exposure to microgravity on the transit back to Earth. This probably represents the greatest challenge with respect to maintaining a safe level of skeletal muscle performance for exploration-class missions.

VII. Gaps

Despite four decades of effort, success in the prevention of spaceflight-induced muscle atrophy and skeletal muscle functional deficits has not yet been achieved in every case, although progress has been made. Gaps in our knowledge have prevented us from implementing a countermeasures program that will fully mitigate the risks of losing muscle mass, function, and endurance during exposure to the microgravity of spaceflight, particularly during long-duration missions. There are also gaps in our knowledge related to working and living in partial-G environments and the effect that wearing an EVA suit has on human performance in such an environment. The countermeasure readiness level for exercise is very high, and developing countermeasures for humans is the highest priority. It is also important to utilize animal models that have the potential to be translated to similar human studies.

The major knowledge gaps that must be addressed by future research to mitigate this risk of loss of skeletal muscle mass, function, and endurance include but are not limited to the following:

- For humans living in a microgravity environment, the optimal exercise regimen, including the mode(s), intensity, and volume needed to minimize or fully mitigate risk, is not known. An appropriate exercise prescription must be developed and validated during spaceflight.
- It is likely that an optimal exercise prescription will include high-intensity resistance exercise. It is important to understand how to periodize and prescribe high-intensity exercise over increasingly longer mission durations without excessively raising injury risk. A high-intensity exercise program may be an effective countermeasure, but it may be difficult or perhaps impossible for some individuals to sustain over longer (1-3-year) mission durations.

- It is important to understand how interruptions in the exercise program can be handled during long-duration exploration programs. Is it acceptable to periodize exercise to provide planned rest periods? How long could crew members go without resistance exercise? What adjunct therapies could be provided in the event exercise must be discontinued?
    - Understanding the balance between muscle protein synthesis and degradation will be helpful in predicting the response to interrupted exercise. It may also help to periodize exercise over long durations. For example, does resistance exercise aemliorate atrophy by also inhibiting NF-kB? This response is proposed to prevent expression of E3 ubiquitin ligases that regulate proteolysis, such as Atrogen-1 and MURF-1. Alternatively, could a nutrient supplement combined with resistance exercise both enhance synthesis via activation of the mTORC1 signaling pathway along with REDD1/2 inhibition, which is an inducer of the ubiquitin-proteosome pathway of proteolysis.
- It is necessary to develop exercise programs that include a balance component to preserve motor control and neuromuscular function in addition to muscle mass and force-generating capability.
- It is unknown whether chronicly unloaded muscle is more vulnerable to musculoskeletal injury (including cartilage and joint) upon reloading in a partial-G environment. Rehabilitation techniques in micro- and partial-gravity are completely unknown.
- The types and functional requirements of exercise hardware and the most comfortable human-to-hardware interfaces needed to minimize or fully mitigate risks are not known. Such hardware is likely to be mission-specific and should be developed and tested using a bed rest environment and then validated in spaceflight.
- Understanding the time course of loss of muscle mass, strength, and function is important to titrate the exercise prescription with the hardware needs for missions of differing durations.
    - It is important to understand whether the dominant mechanism underlying the spaceflight atrophic process is protein degradation via the ubiquiting-proteasome axis or decreased protein synthesis due to loss of ribosomal RNA and pre-mRNA of actin and myosin. This knowledge can inform the choice of pharmaceutical or nutritional supplements as adjuncts to exercise.
- For humans living in partial-G environments, the optimal exercise regimens, including the mode(s), intensity, and volume needed to minimize risk, are not known. Appropriate exercise prescriptions must be developed and validated for partial-G environments.
- It is important to understand the muscle strength, power, and endurance required for optimal exploration mission task performance and develop readiness tests to assess crew members especially after very long transit times, such as those expected with a Mars mission.
- Unloading-induced insulin resistance has been commonly reported in the research literature, although is generally has not been observed to cause major health problems

on 6-month missions. It remains to be determined whether longer duration missions lead to clinically relevant insulin resistance of skeletal muscle.

- A bed rest analog is an efficient way to develop and test exercise prescriptions and hardware. New hardware and exercise protocols could be validated in bed rest and then verified in microgravity using a smaller number of research participants as a resource-sparing strategy.

HRP Baselined Gaps:

M1: What is the current state of knowledge regarding exercise performance?

M2: Characterize in-flight and post-flight muscle performance.

M4: Establish muscle fitness standards for successful completion of mission tasks.

M6: Develop pre-flight and in-flight evaluations to determine if muscle fitness standards are met.

M7: Develop the most efficient exercise program for maintenance of muscle fitness.

M9: Identify and validate exploration hardware for maintenance of muscle fitness.

M14: Identify adjuncts to exercise countermeasures that can be used to better mitigate muscle loss.

M23: Determine if factors other than unloading contribute to muscle atrophy during space flight.

M24: Characterize the time course of changes in muscle protein turnover, muscle mass, and function during long duration spaceflight.

SM7: Determine if there are decrements in performance on functional tasks after long-duration spaceflight. Determine how changes in physiological function, exercise activity, and/or clinical data account for these decrements.

VIII. Conclusion

This report has reviewed evidence from human and animal spaceflight and ground analog data relative to decrements in skeletal muscle mass, strength, and endurance, and the relevant findings have been presented. Data from human spaceflight and ground-based studies are aiding in the determination of the required exercise paradigms but continue to provide an incomplete answer regarding the effective approach for maintaining skeletal muscle function in all human space travelers. With the forthcoming ISS rodent habitat, it will become possible to study important topics (from the list above) using animal models to investigate the relevant molecular

events and signaling and regulatory proteins, and it is this information that will allow a significant change in our approach to maintaining skeletal muscle health and function. Such animal work could eventually lead to novel, likely pharmaceutical, countermeasures to enhance the effectiveness of exercise in humans. While it is certainly possible that exercise alone will be sufficient to protect Mars crew members, it is not certain. A thorough understanding of what exercise will be required for Mars missions and the extent to which supplemental countermeasures will be required or desired will also provide a large Earth benefit in several areas, including rehabilitation, age-related sarcopenia, and efficient exercise programs

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XI. List of Acronyms

1-RM	Repetition maximum test
ACES	Advanced crew escape suit
ANOVA	Analysis of variance
ARED	Advanced Resistive Exercise Device
ASCR	Astronaut Strength, Conditioning and Rehabilitation
CEV	Crew exploration vehicle
CEVIS	Cycle Ergometer with Vibration Isolation and Stabilization
CM	Command Module
CMP	Command Module Pilot
CO2	Carbon dioxide
CSA	Cross-sectional area
DEXA	Dual energy x-ray absorptiometry
DoD	Department of Defense
DSO	Detailed Science/Supplementary Objective
EDOMP	Extended Duration Orbiter Medical Project
EMG	Electromyography
EVA	Extravehicular activities
HR	Heart rate
HS	Hindlimb suspension
iRED	Interim Resistive Exercise Device
ISS	International Space Station
LBNP	Lower-body negative pressure
LEM	Lunar Excursion Module
MHC	Myosin heavy chain
MPS	Muscle protein synthesis
MPB	Muscle protein breakdown
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
mTOR	Mammalian target of rapamycin
MURF	Muscle ring finger protein
NASA	National Aeronautics and Space Administration
O_2	Oxygen
PRD	Program Requirements Document
RED	Resistive exercise device
SERCA II	Sarcoplasmic reticulum ATPase-driven calcium pumps
SLS	Space Life Sciences
SMEAT	Skylab Medical Experiments Altitude Test
SOL	Soleus
SR	Sarcoplasmic reticulum
STS	Shuttle Transport System
TVIS	Treadmill with Vibration Isolation and Stabilization
ULLS	Unilateral lower-limb suspension
VI	Vastus intermedius
VO_2max	Maximal oxygen uptake; aerobic capacity